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THE ANALYST

PROCEEDINGS OF THE SOCIETY FOR ANALYTICAL CHEMISTRY

DEATHS

We record with regret the deaths of

Eric Hucknall
Harry Malkin Mason.

Obituary

HARRY MALKIN MASON

Harry Malkin Mason died suddenly in a nursing home in Harrogate on Monday, May 12th, 1958, aged 71 years.

Mason was a native of Rotherham, and he graduated from Sheffield University in 1907. From 1907 to 1912 he was employed by the well known Sheffield firm of consultant chemists, A. H. Allen and Partners, and he then joined the late G. Rudd Thompson, Public Analyst for Monmouth, where he remained until he joined the R.A.O.C. and served in France until 1919. After demobilisation, he returned to the University and carried out research for the M.Sc. degree. He was appointed chief chemist to James Pascall Ltd. in 1920 and remained with that firm until he joined John Mackintosh & Sons Ltd. in September, 1922, as their first chief chemist.

He established the Research Department of Mackintosh's and directed its activities until January, 1948, when he was compelled by ill health to retire. During the twenty-six years he was in charge at Halifax, a very large volume of work was done under his direction. This included fundamental research on raw materials, processes and products, routine control testing of raw materials, packing materials and the development of new lines.

He had been Chairman of the North of England Section of the Society, and had also been Chairman of the Leeds Section of the Institute of Chemistry. He was a Vice-President of the Society in 1932–33 and 1945–46, and served on the Council in 1930–31 and 1943–44. He served also on the Council of the Institute of Chemistry and on the Committee of the Food Group of the Society of Chemical Industry.

Mason also took an active interest in the work of the Food Industries Research Association. He was a member of the Sugar Confectionery and Chocolate Panels of the Association for twenty-seven years and was Chairman of the former for the whole period and of the latter for five years.

All who knew Mason appreciated his quiet, kindly and unassuming character, and those who were privileged to work with him could not fail to be impressed by his great knowledge and ability both as chemist and administrator. During the last fourteen years of his life he bore the trials of a serious illness with great fortitude and cheerfulness, and up to the last he could always find an apt story to illustrate a point.

He leaves a widow and one son.

A. G. Lipscomb

A Modified Method for the Determination of Polycyclic Hydrocarbons

By B. T. COMMINS

(*Medical Research Council, Dunn Laboratories, St. Bartholomew's Hospital, London, E.C.*1)

A modified method for the determination of polycyclic hydrocarbons is described. The modification involves the separation of the hydrocarbons on alumina columns with *cyclo*hexane, and then the spectrophotometric determination of the hydrocarbon content of each fraction separately over a narrow waveband.

Results of repeated determinations on aliquots of extracted smoke samples are given.

The method has been used to determine the polycylic hydrocarbon content of smoke collected in and around diesel-bus garages.

It is important to realise that the accuracy with which polycyclic hydrocarbons can be determined depends largely on the completeness of their separation. Incomplete separation leads to highly erroneous results owing to the spectra of the hydrocarbons interfering with each other.

Cooper[1] has described a method for determining micro amounts of polycyclic hydrocarbons in samples of soot. The hydrocarbons are extracted with a suitable solvent, separated chromatographically on columns of alumina and determined in the eluted fractions by means of ultra-violet absorption spectrophotometry.

The polycyclic hydrocarbons all have characteristic absorption peaks, and the amounts of hydrocarbons present in the eluted fractions from the chromatographic column are determined by measuring the height of a peak above a base line given by the absorption at two wavelengths on either side of the peak. The peak heights for each polycyclic hydrocarbon are measured and compared with those from solutions containing known amounts of pure hydrocarbon.

Cooper's method involves the measurement of peak heights with wide base lines of up to 20 mμ. I have found that the use of such wide base lines can cause errors in the determination of hydrocarbons, owing to spectral interference by other hydrocarbons and some unidentified substances. To reduce the effect of this spectral interference, base lines as narrow as 8 mμ have been adopted. The effect of this is shown by the results given in Table I. Pure samples of anthracene and pyrene were examined in pure *cyclo*hexane and in *cyclo*hexane containing oil as a contaminant. The use of both wide and narrow base lines gave low recovery from the contaminated solutions, but the error was much smaller when the narrow base line was used.

TABLE I

COMPARISON BETWEEN DETERMINATIONS OF HYDROCARBONS BY USING WIDE AND NARROW BASE LINES IN THE PRESENCE OF OIL AS A CONTAMINANT

Hydrocarbon	Contaminant	Wavelengths used, mμ		Slit width, mm	Optical heights of hydrocarbons determined— in pure *cyclo*hexane (A)	Optical heights of hydrocarbons determined— in *cyclo*hexane - oil mixture (B)	Ratio $\frac{B}{A}$, %
Anthracene	Lubricating oil	366·0, 376·0, 386·0	wide	0·06	0·1500	0·1160	77
		372·0, 376·0, 380·0	narrow	0·06	0·1155	0·1090	94
Pyrene	Gas oil	327·0, 335·0, 343·0	wide	0·30	0·3395	0·2835	84
		332·0, 335·0, 338·0	narrow	0·30	0·2225	0·2135	96

Table II gives the new determining peaks for the polycyclic hydrocarbons. The standard optical heights[2] are calculated as a mean of two or more determinations from different specimens of the hydrocarbon (1:2-benzopyrene was available only from one source).

TABLE II

MODIFIED WAVELENGTHS AND STANDARD OPTICAL HEIGHTS USED FOR DETERMINATION OF POLYCYCLIC HYDROCARBONS

Hydrocarbon	Wavelengths, mμ	Slit width, mm	Standard optical height per μg per ml for 1-cm cells
Anthracene	372·0, 375·8, 380·0	0·06	0·035
Pyrene	332·0, 335·0, 338·0	0·30	0·155
Fluoranthene	285·5, 287·5, 289·5	0·30	0·157
1:2-Benzanthracene	286·5, 288·5, 290·5	0·30	0·073
1:2-Benzopyrene	329·0, 331·5, 334·0	0·20	0·092
3:4-Benzopyrene (I)	381·5, 384·5, 387·5	0·06	0·0325
3:4-Benzopyrene (II)	399·5, 402·5, 405·5	0·04	0·0014
1:12-Benzoperylene	381·5, 384·5, 387·5	0·06	0·043

To assess the reproducibility of results of hydrocarbon determinations by the modified method, several aliquots of soot extracts in *cyclo*hexane were analysed and standard deviations were calculated.

This modified procedure has been applied successfully to the determination of the hydrocarbons in the soot collected from the atmosphere in and around two London Transport bus garages.[3,4]

THE DETERMINATION OF HYDROCARBONS IN SMOKE SAMPLES

All solvents were purified twice by redistillation. Sample 200-ml portions of all the solvents used were evaporated to small bulk, and the residues were tested for the presence of hydrocarbons by spectrophotometry at the absorption peaks given in Table II.

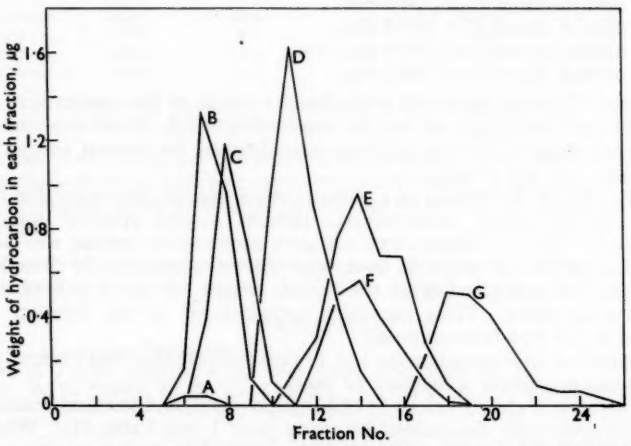

Fig. 1. Typical separation of hydrocarbons in 2·7-ml *cyclo*hexane fractions from a sample of atmospheric soot collected at St. Bartholomew's Hospital in December, 1956: curve A, anthracene; curve B, pyrene; curve C, fluoranthene; curve D, 1:2-benzanthracene; curve E, 3:4-benzopyrene; curve F, 1:2-benzopyrene; curve G, 1:12-benzoperylene

Smoke samples collected on glass-fibre filter sheets were extracted with 300 ml of *cyclo*hexane in acetone-washed Soxhlet thimbles for 3 hours. After extraction, the *cyclo*hexane solutions were heated gently and the volumes were reduced to about 20 ml by drawing filtered air through the solutions. To reduce the volume further, the extracts were allowed to evaporate in small beakers at room temperature and were finally made up to standard volumes of 10 ml or less, depending on the amount of soot extracted. Aliquots of the soot extracts (not greater than 2 ml) were then placed slowly from a pipette on to columns of activated adsorbent.

A suitable adsorbent was prepared by shaking 4 per cent. w/w of distilled water with 100 to 200-mesh alumina type H, obtained from Peter Spence and Sons Ltd., and the alumina columns were prepared by forming a slurry of the adsorbent and *cyclo*hexane, which was then poured into a 1·4-cm diameter Pyrex-glass chromatographic tube to give a column 7·5 cm long. The hydrocarbons on the column were then slowly eluted with *cyclo*hexane and the eluates were collected in 2·7-ml calibrated tubes. Each eluate was examined and the hydrocarbon content was measured in 1-cm silica cells with a Unicam SP500 spectrophotometer. Fig. 1 shows a typical separation of the hydrocarbons obtained during the analysis of a sample of atmospheric soot collected at St. Bartholomew's Hospital in December, 1956.

Some hydrocarbon peaks are not well separated from others, and in such instances the determining wavelengths of the hydrocarbons interfere; special calculation techniques have then to be devised to determine the amounts of hydrocarbons present. An example of this is given by 1:2-benzanthracene and fluoranthene.

When most of the fluoranthene has been eluted, 1:2-benzanthracene begins to appear (it is indicated by the shifting of the maximum at 287·5 mμ) and, as the determining peaks for these two hydrocarbons are very close, it was thought that the most reliable indication of the amounts of the two hydrocarbons present would be given by solving equations involving the measured absorption at four distinct wavelengths. Only the fractions containing a mixture of fluoranthene and 1:2-benzanthracene were analysed by solving equations.

The weights, in micrograms per millilitre of *cyclo*hexane, of the two hydrocarbons present were calculated from the following equations—

$$x = 7{\cdot}0\ (2W_2 - W_1 - W_3)$$
$$y = 46{\cdot}8W_3 + 6{\cdot}5W_1 - 33{\cdot}1W_2 - 20{\cdot}2W_4$$

where x = amount of fluoranthene present in μg per ml,
y = amount of 1:2-benzanthracene present in μg per ml,
W_1 = optical density at 286·5 mμ,
W_2 = optical density at 287·5 mμ,
W_3 = optical density at 288·5 mμ, and
W_4 = optical density at 289·5 mμ.

W_1, W_2, W_3 and W_4 were measured with the slit width of the instrument set at 0·3 mm.

These equations were derived by the standard method of two-component analysis, in which the optical densities at the selected wavelengths for known concentrations of the two hydrocarbons are combined.

Towards the end of the elution of 1:2-benzanthracene, its determination became difficult because some of the other hydrocarbons present caused spectral interference at its determining peak. The 1:2-benzanthracene peak appeared to persist well after the hydrocarbon had been eluted; in order to overcome this interference, the determination of the hydrocarbon was not continued after the optical height for the 1:2-benzanthracene peak was at a minimum value. (This generally corresponded to the fraction containing the maximum amount of 3:4-benzopyrene.)

The separation of 3:4-benzopyrene and 1:12-benzoperylene was generally good; usually only two fractions contained a mixture of both.

3:4-Benzopyrene is eluted before 1:12-benzoperylene, and the first fractions containing 3:4-benzopyrene only were determined by using peak I (see Table II). When the ratio of peak I to peak II was greater than 2·25, 1:12-benzoperylene was then also present, and the excess of this ratio was calculated as 1:12-benzoperylene in accordance with standard practice.[1]

Anthracene was generally present in only minute amounts and was completely eluted from the column before or when the last fraction of pyrene had been collected. In many analyses, interference from an unidentified compound occurred at the determining peak for anthracene towards the end of its elution; since there were only minute amounts present, considerable errors in the determination resulted.

Pyrene and 1:2-benzopyrene were easily determined, as no spectral interference occurred at their determining wavelengths.

Repeated determinations on smoke samples

In order to compare results from samples taken at different times or places, it is necessary to have some idea of their precision. It is often impracticable to carry out more than a

single determination on each sample, and there is then no way of determining the significance of differences between samples. To serve as a guide in these cases, repeated determinations have been carried out on the kinds of material normally dealt with.

One sample, A, comprising 874 mg of atmospheric soot collected at St. Bartholomew's Hospital, London, at intervals between December 12th and 18th, 1956, on glass-fibre filter sheets, was extracted with *cyclo*hexane, and ten determinations were carried out on aliquots of the extract, which was evaporated to small volume and made up to 25 ml in a calibrated flask. Five 1-ml and five 2-ml aliquots of the extract were analysed as previously described. The means and standard deviations of results from the 1-ml and 2-ml aliquots did not differ significantly and in Table III all ten results have been combined. Another sample, B, consisting of 600 mg of atmospheric soot collected at the same site between February 11th and 13th, 1957, on a glass-fibre filter sheet, was divided into equal portions, which were separately extracted and analysed. The *cyclo*hexane extract of each portion was evaporated to small volume and made up to 5 ml in a calibrated flask; 2-ml aliquots of the solution were used for analysis. Results from this series are also shown in Table III. To obtain a measure of precision common to both series, the coefficients of variation have been determined.

TABLE III

RESULTS OF REPEATED DETERMINATIONS OF HYDROCARBONS IN TWO SINGLE SAMPLES OF ATMOSPHERIC SOOT

	Sample A			Sample B		
Hydrocarbon	Mean hydrocarbon found,* μg per g of soot	Standard deviation, μg per g of soot	Coefficient of variation, %	Mean hydrocarbon found,† μg per g of soot	Standard deviation, μg per g of soot	Coefficient of variation, %
Pyrene	105·7	8·3	8	82·4	7·4	9
Fluoranthene ..	108·1	5·0	5	77·0	8·7	11
1:2-Benzanthracene	180·6	24·6	14	139·5	17·5	13
1:2-Benzopyrene ..	77·9	4·3	6	64·3	5·1	8
3:4-Benzopyrene ..	145·6	15·0	10	121·2	6·4	5
1:12-Benzoperylene	84·0	11·2	13	67·3	9·2	14

* Mean of 10 determinations on aliquots of the extract.
† Mean of 8 determinations on equal areas of the filter.

The coefficient of variation is of the order of 10 per cent. for each hydrocarbon. Whether or not the material is divided before or after extraction seems to make no difference. These results lead me to expect single determinations of this type to lie within about ±25 per cent. of the mean value.

I thank Miss P. M. Harrison, late of this Group, who performed most of the analyses, R. E. Waller for invaluable advice and Dr. E. Clar for the gift of a sample of 1:2-benzopyrene.

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Optimum Conditions of the Acid Dichromate Method for Determining Ethanol in Body Fluids

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The rates of reaction at room temperature of potassium dichromate and ethanol have been studied in 5 to 37 *N* sulphuric acid. Optimum acid conditions lie between 18·5 and 23 *N*, and rates of reaction increase with increase in dichromate concentration.

Rates of reaction of likely interfering substances, however, also depend on acid concentration. Therefore, in choosing a method of analysis, it is usually necessary to effect a compromise.

Suitable conditions for the routine determination can be easily attained in Conway microdiffusion cells. The use of these cells provides a simple, rapid and inexpensive method for the determination of ethanol in body fluids, which is free from interference by formaldehyde, acetone or similar volatile substances.

The increasing forensic importance of assessing the degree of intoxication from the analysis of body fluids has stimulated the development of reliable standard methods for specifically determining ethanol. A paper by Kent-Jones and Taylor[1] described two proposed standard methods, both based on the reduction of dichromate solutions by ethanol; in it they emphasised the necessity for strict attention to detail.

TABLE I

METHODS USED FOR THE DETERMINATION OF ETHANOL IN TISSUE

Concentration of sulphuric acid in reaction solution, *N*	Concentration of potassium dichromate in reaction solution, *N*	Method
5	0·025	Kozelka and Hine.[1,3] Steam-distillate from 2 ml of sample is passed through sodium hydroxide - mercuric chloride solution at 100° C and oxidised at 100° C for 20 minutes*
	0·1	Nicloux.[4] Oxidation at 85° C for 1 hour†
	1	Gettler and others.[5,6] Determination of the acetic acid volatilised after oxidation of the steam-distillate at 100° C
10	0·03	Conway.[7] Microdiffusion is complete after 2 hours at room temperature*
	0·1	McNally.[5] Steam-distillate is oxidised at 100° C for 15 minutes* Rochat.[8] Oxidation for 15 to 30 seconds at 100° C†
	0·4	Winnick and others.[9,10] Microdiffusion in Conway units; complete in 10 hours at room temperature*
18	0·005	Cavett.[1,11] Modified Widmark; diffusion complete after 4 hours at 37° C† Harger and others.[5,12] Steam-distillate oxidised at above 100° C†
	0·01	McNally and Coleman.[13] Micro steam-distillate oxidised at 100° C
	0·05 to 0·1	Southgate and others.[14 to 20] Air aspirated for 30 minutes through specimen and absorbers kept at 80° C* Bamford and others.[14,15] Oxidation of steam-distillate above 100° C‡
36	0·05	Widmark.[21] Original microdiffusion method; carried out at 50° C for 2 hours* Scandrett.[22] Special diffusion apparatus; volatilisation at 100° C and oxidation at 50° C; complete in 30 minutes*

* Excess of dichromate is measured iodimetrically.

† Excess of dichromate is measured with standard red ferrous sulphate or by treatment with excess of ferrous sulphate and then titration with potassium permanganate.

‡ Reduction is measured colorimetrically.

A review of textbooks on toxicology shows that the authorities have generally adopted the original Nicloux method[2] based on the reduction of dichromate, but the modifications made have been nearly as numerous as the authorities. Many of these modifications attempt

to achieve complete oxidation of ethanol to acetic acid by acid dichromate, and the reduction in dichromate concentration has been used to calculate the concentration of ethanol in the original specimen. The methods published in the literature are diverse, and often apparently conflicting statements are made about the interference of volatile substances other than ethanol. In Table I are summarised the details of the modifications according to concentrations of sulphuric acid and potassium dichromate in the reacting liquid, and the varying complexity of the different techniques is shown.

Monnier and Rüedi[23] carry out the oxidation in 5 *N* nitric acid for 5 minutes on a steam-bath.

It is indicated in Table I that more detailed knowledge of the mechanism of the dichromate - ethanol reaction might permit considerable simplification of the quantitative method without loss of sensitivity or reliability. In all methods, ethanol is separated from biological fluids and tissues either by dry or steam-distillation, or by diffusion.

A mass of evidence supports the view that acetic acid is the end-point of the oxidation of ethanol by acid dichromate, with the possible formation of acetaldehyde as an intermediate. Widmark[21] has suggested that carbon dioxide is also formed, but the empirical factor adopted in his method differs little from the theoretical factor for conversion to acetic acid, which is the basis of calculation for all other titration methods.

Whereas Bowen and his co-workers[24,25,26,27] have demonstrated that at low acid concentrations the reaction between dichromate and ethanol is essentially photochemical for the first step in the oxidation, Morton[28] has shown that it is thermal with liquids of the following compositions—

	A	B
Potassium dichromate, *N* ..	0·04	0·025
Sulphuric acid, *N*	0·75	2
Ethanol, per cent.	5	1

The effects of acid concentration and temperature have been investigated by Ferrari,[29,30] who found that the quantitative oxidation of ethanol by dichromate depended on the temperature rather than the time, the optimum temperature being 70° C. He also found that the sulphuric acid concentration bore an algebraic relationship to the rate of reaction, the most favourable concentrations being 12 *N* and higher.

Kratz and Plämbock[18] claim that diethyl ether, ethyl chloride, acetone and chloroform give substantial "apparent ethanol" figures by the Widmark[21] method at 50° C, but low values by the Liebesny[17] method at 80° C, and they attribute the difference to the effect of temperature. Other substances stated in the literature to interfere with the determination of ethanol in body fluids include formaldehyde, acetaldehyde, salicyclic acid and acetoacetic acid and its salts.

Experimental

Rates of reaction of potassium dichromate with ethanol or likely interfering substances were determined in acid solutions of various concentrations to provide information on the most suitable conditions for the oxidation of ethanol. The rates of reaction were determined in test-tubes at room temperature (about 20° C).

Loss by volatilisation—

Equivalent amounts of 1 per cent. v/v ethanol and 2 per cent. v/v acetaldehyde were added to 10 ml of 0·05 *N* potassium dichromate in 10 *N* sulphuric acid in open tubes, and the colours were compared visually at intervals with standards. These standards were prepared in stoppered tubes 24 hours beforehand and contained 10 ml of 0·05 *N* potassium dichromate and 0 to 1·0 equivalents, at intervals of 0·1 equivalent, of 1 per cent. v/v ethanol. The results are shown in Fig. 1.

Although the high volatility of acetaldehyde and ethanol could be expected to cause difficulty in quantitative work, the experimental results in Fig. 1 show that the rate of reduction of 0·05 *N* potassium dichromate by acetaldehyde is about six times faster than by ethanol and that the losses by volatilisation are negligible.

Effect of light—

The rates of reaction of ethanol and 0·05 *N* potassium dichromate in sulphuric acid solutions of concentrations from 5 to 37 *N* were found to be substantially the same in daylight

as in darkness, which confirms Morton's conclusion that, at high acid concentrations, the reaction is thermal and not photochemical.

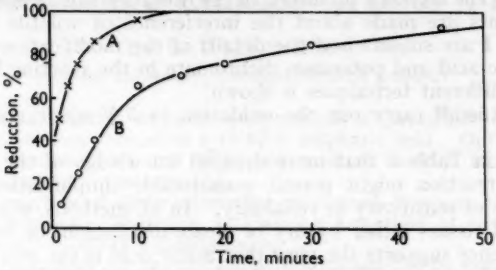

Fig. 1. Rates of reduction of 0·05 *N* potassium dichromate in 10 *N* sulphuric acid at 20° C: curve A, acetaldehyde; curve B, ethanol

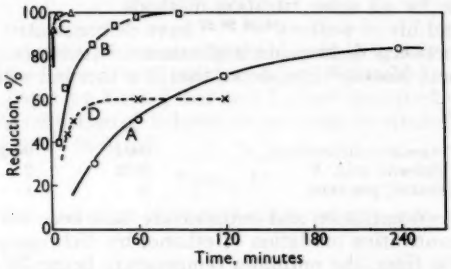

Fig. 2. Reduction of 0·05 *N* potassium dichromate in sulphuric acid of different concentrations by ethanol at 20° C: curve A, 5 *N*; curve B, 10 *N*; curve C, 18·5 *N*; curve D, 37 *N*

EFFECT OF ACID AND DICHROMATE CONCENTRATION—

Although the temperatures of oxidation given in Table I range from room temperature to above 100° C, most methods require the higher temperature, which often involves additional steps or complicated apparatus. Rates at 20° C were determined for the reduction of 10-ml portions of acid dichromate solutions by equivalent amounts of ethanol under various conditions of acidity and dichromate concentration. The reduction of dichromate was carried out in test-tubes, and, in some parallel tests, in Conway diffusion cells with smaller amounts of reactants. At intervals, a portion of the reacting liquid was removed from the test-tube and the residual dichromate was determined iodimetrically. For 37 *N* acid solutions, *i.e.*, concentrated sulphuric acid, the reaction mixture was added to ice-cold water containing

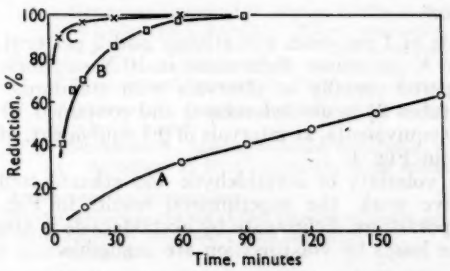

Fig. 3. Reduction of different concentrations of potassium dichromate in 10 *N* sulphuric acid by ethanol at 20° C: curve A, 0·005 *N*; curve B, 0·05 *N*; curve C, 0·5 *N*

iodide to prevent completion of the reaction, which occurs when the concentrated acid solutions are diluted with water alone. The rates of reaction are shown in Figs. 2, 3 and 4.

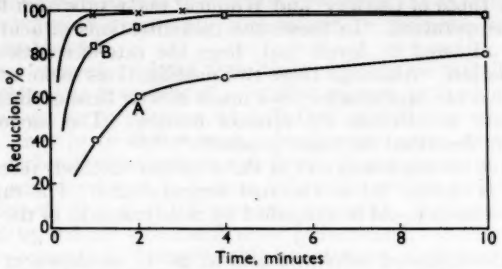

Fig. 4. Reduction of different concentrations of potassium dichromate in 18·5 *N* sulphuric acid by ethanol at 20° C: curve A, 0·005 *N*; curve B, 0·05 *N*; curve C, 0·5 *N*

Colorimetric comparison of the rates of reaction of 0·05 *N* potassium dichromate and ethanol at intermediate acidities showed little difference at sulphuric acid concentrations of 18·5 and 23 *N*, but the rates at 14 and 27 *N* were significantly slower.

Figs. 2, 3 and 4 show that 0·05 *N* potassium dichromate was completely reduced at room temperature in 5 and 90 minutes when the acid concentrations were 18·5 and 10 *N*, respectively. The reduction of 0·5 *N* potassium dichromate in 18·5 *N* sulphuric acid was extremely rapid, and even in 10 *N* acid the reaction was complete in 30 minutes. On the other hand, 0·005 *N* potassium dichromate solutions were reduced very slowly at 20° C, even in 18·5 *N* acid. When the reaction was carried out in concentrated sulphuric acid there was only partial reduction of the dichromate, but, if this partly reduced solution was diluted with water, reduction proceeded to completion, the rate depending on the degree and method of dilution.

The quantitative reduction of dichromate by ethanol can therefore be achieved at room temperature in a reasonable time if the minimum concentrations of acid and dichromate are 10 and 0·05 *N*, respectively.

INTERFERING SUBSTANCES—

The reducing activity of interfering substances was studied by adding 1-ml portions of aqueous solutions of the substances to 10-ml portions of 0·05 *N* potassium dichromate in 10 or 18·5 *N* sulphuric acid and setting the reaction mixtures aside in stoppered test-tubes at room temperature. After suitable intervals, the amount of reduction was determined by visual comparison with previously prepared standards of acid dichromate and appropriate amounts of ethanol. The results are shown in Table II.

TABLE II

EFFECT OF 1-ml PORTIONS OF VARIOUS SUBSTANCES ON 10 ml OF 0·05 *N* POTASSIUM DICHROMATE AT ROOM TEMPERATURE

Substance	Concentration of substance, %	Effect on reduction of 0·05 *N* potassium dichromate in 10 *N* sulphuric acid	Effect on reduction of 0·05 *N* potassium dichromate in 18·5 *N* sulphuric acid
Formaldehyde ..	1 w/v	Reduction complete in 3 minutes	Reduction complete in 1 minute
Acetaldehyde ..	1·5 v/v	Reduction complete in 15 minutes	Reduction complete in 2 minutes
Methanol	0·76 v/v	Reduction complete in 60 minutes	Reduction complete in 2 minutes
Ethanol	0·725 v/v	Reduction complete in 90 minutes	Reduction complete in 5 minutes
Ethyl acetoacetate	1 v/v	Reduction complete in 2 hours	Reduction complete in 15 minutes
Sodium salicylate..	1 w/v	Reduction almost complete in 2 hours	Reduction complete in 30 minutes
Diethyl ether* ..	1 v/v	20 per cent. reduction in 24 hours	Reduction complete in 30 minutes
Acetone	1 v/v	Insignificant reduction in 24 hours	10 per cent. reduction in 8 hours
Ethyl chloride ..	1 v/v	No reduction in 24 hours	10 per cent. reduction in 24 hours
Chloroform ..	1 v/v	No reduction in 24 hours	Insignificant reduction in 24 hours
Methylamine ..	1 v/v	No reduction in 24 hours	No reduction in 24 hours

* Under the same conditions, 0·05 *N* potassium dichromate in 14 *N* sulphuric acid is almost completely reduced by diethyl ether in 8 hours.

Discussion of results

It is shown in Table I that, of the fourteen distinctly different modifications of the Nicloux method, only those of Conway[7] and Winnick[9] make provision for the determination of ethanol at room temperature. In these, the concentrations of acid and dichromate are at, or can be easily adjusted to, levels that, from the rate-of-reaction curves, will ensure reasonably rapid oxidation. Although these two modifications make use of the same micro-diffusion apparatus, that of Conway achieves a much shorter time of diffusion by the addition of potassium carbonate to increase the ethanol tension. The use of Conway units for determining ethanol is described in other papers.[31,32,33,34]

In the other twelve modifications and in three further methods recently described,[35,36,37] oxidation of ethanol is carried out at elevated temperatures. The rate-of-reaction curves suggest that some procedures could be simplified by re-adjustment of the acid and dichromate concentrations.

The reaction in concentrated sulphuric acid at 20° C, as shown in Fig. 2, is surprising in that quantitative reduction of the dichromate does not occur, as it does in the more dilute solutions. This anomaly does not arise with 27 *N* acid solutions, in which reduction follows the pattern of 18·5 *N* solutions. It is probable that, in concentrated acid, side reactions take place with the formation of compounds resistant to further oxidation, but that addition of water to the reaction mixture later results in hydrolysis of these compounds and the reaction then proceeds normally. The past success of the Widmark[21] and the efficacy of the Scandrett diffusion procedure[22] evidently depend on the combined effect of initial dilution of the surface layers of the absorbing liquids and subsequent dilution with water before the addition of iodide.

It is shown in Table II that acid concentration has as much influence on the oxidation of likely interfering substances as on the oxidation of ethanol. It is probable that temperature and dichromate concentration, shown by Ferrari[29,30] and myself, respectively, to be important for ethanol, are also major factors in determining the optimum conditions for oxidation of interfering substances.

Determination of ethanol

The development of a simple, reliable, rapid and specific method for the determination of ethanol must therefore depend on a compromise between rate of reaction and freedom from interference. In practice, the size of sample available and the level of ethanol normally encountered in the body fluids of intoxicated people limit the concentration of dichromate to about 0·05 *N*.

The slow rates of reaction at room temperature of 0·05 *N* potassium dichromate in 10 *N* sulphuric acid with diethyl ether, acetone, ethyl chloride, chloroform and methylamine compared with that of ethanol indicate that these conditions should provide a suitable basis for the determination of ethanol in body fluids by a diffusion technique.

Microdiffusion techniques—

The microdiffusion cells described by Conway[7] provide a simple apparatus in which suitable conditions for the oxidation of ethanol can be achieved conveniently. In my experience, it is essential to use a saturated solution of potassium carbonate or the solid itself as recommended by Conway to ensure that diffusion of ethanol into and its oxidation by the acid dichromate in the centre compartment are complete in 2 hours.

Aqueous solutions containing 1 per cent. v/v of acetone, chloroform, ethyl chloride, diethyl ether or methylamine, either alone or with known amounts of ethanol, were examined by the Conway procedure, 0·05 *N* being used in place of the 0·03 *N* dichromate recommended by Conway.[7] The results showed that these substances did not give an "apparent ethanol value" and did not interfere in the ethanol determinations when diffusion was allowed to take place for 2 hours.

Although when added directly to the oxidising mixture acetoacetic ester reduces the dichromate in 2 hours and in 15 minutes at acid concentrations of 10 and 18·5 *N*, respectively, the interference in the diffusion procedure was shown to be due only to the ethanol liberated on hydrolysis by the saturated potassium carbonate solution. Acetoacetic acid and its salts, therefore, do not affect the significance of ethanol determinations made by the Conway microdiffusion procedure. Other acids, such as salicyclic acid, are likewise converted into non-volatile salts by potassium carbonate.

Formaldehyde, acetaldehyde and methanol reduce acid dichromate solutions more quickly than does ethanol. I found that formaldehyde did not interfere if immediately mixed with the potassium carbonate in the Conway procedure. The alkalinity of the saturated potassium carbonate solution apparently is sufficient to promote polymerisation or condensation of the formaldehyde.

As this experience seemed to differ from that of Feldstein and Klendshoj,[38] the effect of formaldehyde was further investigated. Schiff's reagent in 1-ml portions was found to develop a distinct colour in 10 minutes when 0·1 ml of 0·01 per cent. formaldehyde, *i.e.*, 0·01 mg, was added directly to the reagent. In Conway units, however, when 0·5 ml of saturated potassium carbonate was used in the outer cell and 1·0 ml of Schiff's reagent in the inner, a faint colour was developed in 1 hour only when the concentration had been raised to 0·5 mg of formaldehyde (0·1 ml of 0·5 per cent. solution). Without potassium carbonate, the colour developed by 0·5 mg was much more pronounced. Feldstein and Klendshoj do not state at what concentrations formaldehyde interferes in their method for methanol and, further, their dilution of the saturated potassium carbonate solution is greater than in the Conway technique for ethanol. It seems evident, therefore, that differences in concentration of formaldehyde or dilution of the potassium carbonate may account for the variation in experience, and, in practice, if formaldehyde interferes in the Conway procedure it will be serious only when the concentration is above 500 mg per 100 ml of blood or urine. Such high concentrations are unlikely to occur in normal cases of intoxicated persons involved in motor accidents.

Acetaldehyde does not behave similarly to formaldehyde and is not quantitatively removed at room temperature with either Scott-Wilson's alkaline mercuric cyanide reagent or alkaline mercuric chloride. If acetaldehyde is suspected, its presence can be established by its rate of reaction, formation of aldehyde resin in the centre well of a Conway unit or by Legal's nitroprusside test (see Autenrieth and Warren[39]). It can be quantitatively measured by an adaption of Conway's microdiffusion method for lactic acid.[7]

In interpreting the coexistence of acetaldehyde and ethanol, it is pointed out that there is some evidence[40,41] that acetaldehyde is a primary product of the metabolism of ethanol.

The absence or presence of methanol in a specimen can be established by carrying out a replicate Conway diffusion by the method of Feldstein and Klendshoj.[38]

The simplicity and specificity, together with the robustness, relatively low cost and ease of cleaning of the apparatus, justify the use of the Conway procedure, especially when many specimens are handled or when the amounts of body fluids available are strictly limited. It has the added advantage of permitting replicate determinations to be made at the same time.

The apparatus also provides convenient methods for the identification and determination of substances, such as acetaldehyde or methanol, likely to interfere in ethanol determinations, and also for the determination of other volatile poisons, such as acetone, ether, formaldehyde, chloroform and carbon monoxide.

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o-Dithiols in Analysis

Part VII.* Toluene-3:4-dithiol as a General Analytical Reagent in Qualitative Analysis

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The properties of toluene-3:4-dithiol are closely similar to those of hydrogen sulphide, but it possesses advantages over the latter, and has much to commend it as a general purpose reagent. Its metallic derivatives, formed in acid solutions, show marked colour contrasts and are precipitated in order, often with clean separation. The order of precipitation is given for 34 cations. For many thiophilic elements, toluene-3:4-dithiol, under suitably chosen conditions, is a specific or highly selective reagent. Applications of toluene-3:4-dithiol in elementary qualitative analysis are discussed.

Toluene-3:4-dithiol and hydrogen sulphide

Toluene-3:4-dithiol (dithiol) may be regarded as an organic analogue of hydrogen sulphide, which it resembles closely in many ways. This resemblance extends even to the tendency of the arsenious mercaptide, like the sulphide, to form a colloidal solution[1] and to the fact that nickel and cobalt mercaptides, like the sulphides, are only precipitated in dilute acid solutions, but, once precipitated, will not dissolve in more concentrated acid. Likewise, the product of oxidation resembles sulphur in appearance, and, like the latter, is soluble in carbon disulphide, but in few other solvents. It may be added that sulphur also is soluble in an alkaline solution of dithiol as well as in sodium sulphide solution.

Analytically, dithiol possesses advantages over hydrogen sulphide—its smell is not objectionable, it may be added dropwise and its complexes coagulate rapidly and show great variety in colour. Also, the selectivity of its reactions gives dithiol a claim to be regarded as a multi-purpose reagent. Lack of interest in dithiol hitherto may be ascribed (*a*) to its instability, and (*b*) to the multiplicity and consequent mutual interferences of its reactions.[2] It has been shown[3] that the first difficulty can be overcome by generating the reagent, as

* For details of previous parts of this series, see reference list, p. 402.

required, from diacetyltoluene-3:4-dithiol (diacetyldithiol) or the zinc complex of toluene-3:4-dithiol (zinc dithiol). In this paper it is shown, *inter alia*, that, despite the many colour reactions given by dithiol, interference between these reactions is rarely encountered if advantage is taken of the wide differences in the solubilities and conditions of formation of the mercaptides.

Separation of Cations by Dithiol

When dithiol is added dropwise to hot acid solutions containing thiophilic cations, the elements are precipitated as insoluble complexes. Coagulation is usually very rapid and a teat pipette can often be used directly to remove the supernatant liquid. The mercaptides can also be removed by shaking with a heavy organic solvent, such as ethylene dichloride. Even when they are not dissolved by the solvent, they are preferentially wetted and the warm supernatant liquid is left clear and colourless. More dithiol can then be added and, since fresh precipitation occurs in the upper clear solution, the point at which precipitation is complete can be easily seen. If several metals are present, they are precipitated in a definite order. Since the colours of the complexes are usually highly distinctive, it is often possible to effect extremely rapid and quite sharp separations of metals in this way.

When dithiol is so used, interference between its various reactions is limited, and, when it occurs, can often be circumvented. If, therefore, dithiol can be used for the detection of an element, the fact that other elements also react with it need not, in principle, prevent its use as a specific, or at least highly selective, reagent for the element in question.

Hence, if a mixture of mercury^{II}, copper, tin^{II} and lead chlorides (0·001 to 0·005 *M* of each in warm *N* hydrochloric acid) is treated as described, a yellow (mercury^{II}) complex separates first, and is taken up by the ethylene dichloride in which it is insoluble. A black (copper) complex is then formed, and, at a certain point in the addition, the colour of the freshly formed precipitate changes suddenly to the red of the tin complex, which gives a yellow solvent solution (see p. 398). Finally, after the acidity has been reduced to 0·1 to 0·5 *N*, the brilliant yellow lead complex is precipitated. On each occasion that the freshly formed precipitate takes on a new colour, the upper layer is removed with the aid of a teat pipette and the lower layer is washed with water or dilute acid and the washings are discarded.

In this way 4 to 6 heavy-metal constituents of a mixture can often be identified and rough separations made within 50 to 90 seconds. When it is desirable to have confirmatory tests, these are easily carried out on the "fractions" obtained by decomposing the complexes.

The method has its limitations. Sharp separation of two metals is impossible if the complexes are insoluble in concentrated hydrochloric acid, if they are of comparable solubility or if they are not distinctively coloured. Difficulties may arise also if one complex has a low melting-point and can dissolve the complex of another metal—as with antimony and tin in hydrochloric acid. Many of these difficulties, however, disappear if the elements are separated into the usual analytical groups.

General Procedure—

Dilute the solution until the concentration of no single cation much exceeds 0·02 to 0·03 *M*. To 3 or 4 ml of the solution, suitably acidified, add 1 ml of ethylene dichloride; then warm and add dithiol (0·04 to 0·1 *M*, prepared as described in Part IV of this series[4]) dropwise with shaking. When no more coloured precipitate forms, or the colour of the freshly formed precipitate changes, withdraw the upper layer with the aid of a teat pipette and transfer it to a second tube. Continue the addition of dithiol after adding more ethylene dichloride (see Note) and, if desired, reducing the concentration of acid. Repeat the operations until further addition of dithiol no longer gives a coloured precipitate.

Decompose each fraction in turn by boiling with hydrochloric acid and, if necessary, a few drops of bromine water. The chlorides are then left in solution, a few specks of coagulated dithioldisulphide remaining insoluble.

Note—It is sometimes advantageous to coagulate the precipitate by boiling before the addition of ethylene dichloride in each unit operation.

The Order of Precipitation of Cations and the Properties of the Complexes

Thiophilic cations can be arranged in a list such that, if any two elements in the list are present in a solution and dithiol is added, the first-named in the list is precipitated preferentially. (The solutions used in this investigation were 0·001 to 0·005 *M* and the acidity was varied as indicated. Ethylene dichloride was used as the solvent.)

CATIONS PRECIPITATED IN COLD OR WARM HYDROCHLORIC ACID GREATER THAN 10 *N*—

Palladium^{II}—Brick-red precipitate that is soluble in ethylene dichloride.

Tellurium^{IV}—Intense yellow precipitate that is soluble in ethylene dichloride. When warmed with excess of dithiol, the solution darkens, becoming red or purple, and rapidly deposits a black precipitate (tellurium?). The drops of solvent then show a metallic lustre that is not given with any other element.

Gold—Characteristic brownish pink precipitate that is sparingly soluble in ethylene dichloride. If the suspension in the solvent is evaporated to dryness in a tube and the residue is heated, it turns an intense purple; on very strong heating the glass becomes gilded in patches.

Selenium^{IV}—Yellow oily precipitate that is soluble in ethylene dichloride to give a yellow or yellowish green solution.

Tungsten^{VI} *and rhenium*^{VII}—No indication of the separation of these cations was observed. The blue tungsten complex forms slowly.[5,6] If, in a rather more dilute acid, a little osmic acid is added first, followed, drop by drop, by dithiol, the presence of tungstate inhibits the development of the osmium colour, which suggests that a colourless tungsten complex may be formed first. When dithiol is used as a test for tungsten, a rapid rate of reaction can best be achieved by starting in an alkaline solution if elements that are likely to interfere (especially platinum and molybdenum^{VI}) are absent. If the tungstate solution is made alkaline and boiled with excess of dithiol for a few seconds and then acidified, the blue colour appears almost immediately. Rhenium gives a green precipitate that is soluble in ethylene dichloride.[5,7]

CATIONS PRECIPITATED IN 6 TO 8 *N* HYDROCHLORIC ACID—

Osmium^{VIII}—A deep purple precipitate forms rather slowly; it is soluble in ethylene dichloride to give a purple solution, which, on addition of pyridine, becomes greenish black (0·5 μg per ml of osmium was easily detected).

Rhodium^{III} *or rhodium*^{IV}—No precipitate is given with dithiol unless a strong reducing agent, such as hydroxylamine or hypophosphorous acid, is also present, when a yellowish brown precipitate forms slowly. (Precipitation is rapid in 5 *N* acid.) The precipitate melts in the boiling acid, solidifies on slight cooling and dissolves in ethylene dichloride giving an intensely brownish yellow solution.

Arsenic^{III}—Very pale yellow precipitate that melts in the hot liquid and forms an emulsion.[1] Arsenic^{V} does not react, but is rapidly reduced.[1]

Iridium and ruthenium—Owing to the similarity in colour of the complexes and the slowness of the reactions, the order of formation was not determined. Iridium gives a jet-black precipitate that is soluble in ethylene dichloride to give an intensely brown solution, which rapidly blackens with deposition of solid. Ruthenium gives a pale brown precipitate that is insoluble in ethylene dichloride. Both precipitates are formed rather slowly in concentrated acid, but more rapidly in 5 *N* acid. The colours of solutions of iridium^{III} and ruthenium^{III} are at first discharged as a result of reduction by dithiol.

Germanium^{IV}—White precipitate, which forms a milky suspension[1] and coagulates slowly to form pale yellow particles.

Copper^{I}—Grey-black precipitate of low colour intensity (see copper^{II}).

Mercury^{II}—Pale yellow precipitate that is insoluble in ethylene dichloride.

CATIONS PRECIPITATED IN DILUTE ACIDS—

The approximate maximum concentration of boiling hydrochloric acid at which precipitation occurs is given in parentheses. At lower temperatures, precipitation may occur in much more concentrated acids. Acetate buffer was used to determine the order below cobalt.

Silver—Bright yellow precipitate that is insoluble in ethylene dichloride. (3 to 3·5 *N*.) (In dilute acid an orange complex is formed.)

Antimony^{III}—Orange precipitate that melts to a yellow oil when heated. (About 3 *N*.) For the reaction of antimony^{V}, see Part III of this series.[3]

Tin—Red precipitate. (3 *N*.[8]) The compound is evidently a complex of both tin^{II} and tin^{IV} and has the formula $H_4Sn_2(C_7H_4S_2)_5$. The corresponding compound prepared from 4-chloro-1:2-dimercaptobenzene gave a crystalline analytically pure tetrapyridinium salt.[9] This formula is in accordance with Pollak's[10] analyses for carbon and hydrogen in the compound $H_4Sn_3(C_6H_4S_2)_5$ and with Ovenston and Kenyon's observation that the ratio of dithiol residues to tin atoms in the dithiol complex is approximately 2·4 to 1.[11] The presence of both tin^{II} and tin^{IV} is suggested by the fact that a saturated solution of stannous chloride in dilute hydrochloric acid does not give a red precipitate with dithiol, but does so immediately if a stannic compound is added.

The red compound is insoluble in solvents—the yellow solutions usually produced are of another compound, which gives the red compound when heated (see tin^{IV}).

Copper^{II}—Black precipitate that is very slightly soluble in ethylene dichloride to give a greenish brown solution. (About 2 to 2·5 *N*.) The complex contains both Cu^{I} and Cu^{II}. If Cu^{I} only is present, a grey-black precipitate forms. Both complexes give the same colour reactions with pyridine and with sodium hydroxide and excess of dithiol.[3]

Molybdenum^{VI}—Intense green precipitate that is soluble in ethylene dichloride. The aqueous layer becomes blue on addition of a concentrated solution of ammonia. The green complex forms rapidly in 2 *N* acid, but traces are formed, sometimes slowly, at all acidities from 10 *N* to acetate buffer. Sharp separation of molybdenum from other elements is, accordingly, often poor (see later, p. 400).

Platinum^{II}—Platinum^{IV} is slowly reduced to platinum^{II}, which gives an intensely coloured violet precipitate that is soluble in ethylene dichloride to give a violet solution. (About 1·5 *N*.)

Bismuth—Orange-red precipitate[12] that is soluble in ethylene dichloride and slightly soluble in boiling dilute hydrochloric acid, the latter reversibly giving a colloidal orange solution on cooling. The bismuth complex cannot be completely removed by a single extraction with ethylene dichloride. (About 1·5 *N*.)

Cobalt—Jet-black precipitate that is insoluble in ethylene dichloride. (About *N*.) Excess of dithiol in acetate buffer gives a blue colour to the solution[3] and the formation of the blue compound may interfere with the separation of cobalt from the cations listed below.

Lead—Bright yellow precipitate that is soluble in ethylene dichloride. (About 0·5 *N* cold acid or 0·1 *N* boiling acid.)

Cadmium—White precipitate that is insoluble in ethylene dichloride. (Acid slightly less concentrated than for lead.) The complex rapidly gives yellow cadmium sulphide when heated strongly.

Thallium—Pale orange precipitate that is soluble in ethylene dichloride. (About 0·01 *N* in cold.)

Nickel—Greenish to grey precipitate that is insoluble in ethylene dichloride. The colour varies with the conditions.

Zinc—White precipitate that is insoluble in ethylene dichloride.

Iron^{II}—Intense black precipitate that is insoluble in ethylene dichloride. Excess of dithiol produces a red solution.[3] Separation from zinc is not sharp.

Tin^{IV}—Yellow precipitate that is soluble in ethylene dichloride to give a yellow solution. The solution reddens on standing, or when heated or when mineral acid is added.

Tin^{II}—White precipitate. The solution reddens as with the tin^{IV} complex.

Gallium—Precipitate forms as a white milky suspension, which fails to coagulate. Causes ethylene dichloride to form a stable emulsion. (No other element behaves similarly.)

Indium—White precipitate, which coagulates slowly to give a pale yellow precipitate that is soluble in warm ethylene dichloride.

Chromium^{III}—No precipitate, but its presence inhibits the manganese reaction.

Manganese—See Part III of this series.[3]

Vanadium—Strong colour in presence of pyridine, but not ammonia. See Part III of this series.[3]

The complex salts of mercury, cadmium and zinc, containing 2 molecules of dithiol residue to one of metal,[13] are instantly decomposed by water and are not formed in its presence.

Of the elements in the list, palladium^{II}, tellurium^{IV}, selenium^{IV}, rhenium^{VII} and iridium also give precipitates with diacetyldithiol.[4]

Clean separations with the less soluble complexes that form in concentrated hydrochloric acid are not possible, since the extremely low solubilities make the reactions irreversible. Thus, when once the osmium complex has separated, the addition of a tungstate does not cause it to re-dissolve.

Group 1 elements

The order of precipitation is tungsten^{VI}, mercury^{II}, silver, lead and thallium^{I}. (In hydrochloric acid solution sufficiently concentrated to dissolve silver chloride, mercury^{I} is converted to mercury^{II}.) Tungsten^{VI}[14] and certain rare cations[4] may first be removed, if desired, by means of diacetyldithiol. Separations of mercury from silver and of silver from lead are easily carried out. In acetate buffer lead and thallium do not give a clean separation, owing to the similarity in the colours of the precipitates. In 0·3 *N* mineral acid, however, only lead is precipitated in a warm solution (5 μg per ml can be detected). If sodium acetate is then added, the thallium complex separates (for confirmatory test, see Part III of this series[3]). The reactions for silver, lead and thallium are characteristic. If sodium hydroxide in excess is added to a solution, which is then filtered, and zinc dithiol is added, a yellow precipitate is formed only in the presence of lead or thallium. (With thallium, the precipitate first formed is orange, but it rapidly becomes yellow.)

Silver may be detected in 2 to 4 *N* nitric acid by adding zinc dithiol and warming; the mixture suddenly becomes bright yellow. In this reaction, oxidising agent and cation compete for dithiol and only palladium^{II}, silver, mercury, platinum (yellow colour, not violet), gold and tellurium react.

Group 2A elements (copper group)

The order of precipitation of the elements of group 2A is ruthenium^{II}, mercury^{II}, copper, platinum^{II}, bismuth, lead, cadmium and thallium.

Ruthenium causes the solution to darken slowly before mercury is precipitated. Ruthenium^{III} is normally reduced with loss of the claret colour to ruthenium$^{II(?)}$, but if mercury is also present a red precipitate, possibly akin to the red of ruthenium, sometimes forms. The conditions for its formation could not be ascertained. The separation of ruthenium from copper is sharp.

If platinum^{II} (or platinum^{IV}, the reaction being slower) and bismuth are present together, the red bismuth colour forms first. When the solution is shaken with ethylene dichloride and warmed, the supernatant liquid rapidly becomes violet and the red colour of the bismuth

complex is discharged, the lower solvent layer also becoming violet. When all the platinum has reacted, the red bismuth complex appears a second time on the addition of more dithiol, so that platinum and bismuth are easily separated. For lead and thallium, see group 1 elements.

Dithiol affords a selective reagent for most of these elements. The pale yellow mercury^{II} complex, formed in concentrated acid, has a characteristic appearance and tends to "climb" the sides of the tube. Owing to its lack of intense colour it is difficult to identify in presence of much dithioldisulphide. However, if it is suspended in the solvent it becomes yellow and will darken when treated with hydrogen sulphide. Alternatively, it may be decomposed with hydrochloric acid and bromine water. A trace of a mercury salt may then be detected immediately by its power of discharging the blue colour of the solution obtained by adding a *trace* of zinc dithiol to pyridine containing a small amount of a cobalt salt.

Only iridium, osmium, ruthenium (grey) and copper give black precipitates in hot 2 *N* acid. The first three of these are readily separated by means of dithiol in a more concentrated acid, and copper, in the copper complex suspended in ethylene dichloride, may be confirmed by the addition of a concentrated solution of ammonia or sodium hydroxide and zinc dithiol, which gives an intense orange solution. (With mercury the aqueous layer is colourless and with bismuth it is pure yellow.) Platinum^{II} cannot be confused with copper, owing to the slowness of its precipitation.

The intense mauve-violet colour of the platinum complex is characteristic of the element (2 μg per ml was detected). At great dilution, 2 minutes should be allowed for the development of the colour. The violet solution in the solvent retains its colour when boiled with concentrated hydrochloric acid (distinction from copper, bismuth, mercury, etc.). If a solution containing platinum^{II} or platinum^{IV} is first made alkaline, and then dithiol is added and the solution is boiled and acidified, the violet complex is formed immediately (compare tungsten).

The bismuth complex is slightly soluble in hot aqueous solution, and this makes complete separation from lead more difficult. However, bismuth is easily detected in presence of much lead; if excess of alkali is added and the solution filtered, zinc dithiol gives a yellow precipitate with the filtrate, as in group 1.

REACTIONS IN MOLTEN UREA: DETECTION OF BISMUTH—

The reaction of dithiol with bismuth (1 μg per ml was detected) was recommended as a test for this element by Miller and Lowe.[12] The test, however, was not specific, but may easily be made so by the use of urea.

The order of precipitation of the mercaptides is changed if molten urea is used in place of water as a solvent,[15] bismuth and then lead being precipitated before the other group 2 metals. If, further, a little potassium cyanide is added, the test apparently becomes specific. The only elements found to give colours were uranium, as uranyl salt (very pale yellow), bismuth (intense dark red), tellurium (yellow) and lead (intense yellow), and the elements reacted in this order. The addition of water at once bleached the uranyl complex (uranium gives no coloured product with dithiol in aqueous solution), but not that of bismuth.

In this and similar reactions it has not been found necessary to use anhydrous urea. It it only necessary to add urea crystals in excess and to warm the mixture until the urea melts.

Procedure for the detection of bismuth—To 0·2 ml of the test solution acidified 4 to 6 *N* in hydrochloric acid, add 1 to 2 g of solid urea and heat to melt the urea. (If much bismuth is present a white precipitate forms, but does not interfere with the reaction.) Add 1 drop of 10 per cent. potassium cyanide solution, and then add dithiol dropwise. Cool the solution slightly; a dark red colour, unchanged by addition of water, indicates the presence of bismuth.

In absence of bismuth, lead gives a yellow precipitate.

GROUP 2B ELEMENTS (ARSENIC GROUP)

When dithiol is added to *N* hydrochloric acid containing arsenic^{III}, antimony^{III}, molybdenum^{VI} and tin^{II}, the elements are precipitated substantially in the order stated, but clean separations are not achieved. In *N* sulphuric acid the order changes to arsenic^{III}, antimony^{III}, tin^{II} and molybdenum^{VI}, and the separation is better with much less masking of the tin colour. However, colour contrasts are too poor for good separation and the formation of the molybdenum complex is not readily reversible.

If 5 *N* hydrochloric acid is used to dissolve antimony and tin sulphides, leaving the sulphides of arsenic and molybdenum, dithiol may be used to detect arsenic[1] and molybdenum[5]

in the residue. In the solution, antimony^{III} forms the low-melting orange compound $C_7H_6S_2SbCl$, in which the tin complex appears to be soluble, so that separation cannot be effected. However, if chlorides are present, antimony and tin may be detected together if the acidity is first reduced by the addition of sodium acetate. Addition of solvent then removes the antimony complex, leaving, apparently, a colourless water-soluble tin - dithiol complex in solution, which gives the red compound on acidification (12 μg per ml of tin in presence of 0·4 mg of antimony in 1 ml were detected).

Procedure for the detection of tin^{II} *in presence of antimony in hydrochloric acid solution*—To 2 ml of the test solution add sodium acetate crystals in excess and 0·5 to 1·0 ml of ethylene dichloride. Heat to the boiling-point and add dithiol dropwise with shaking until a further drop no longer produces a yellow colour in the aqueous layer. (The antimony complex dissolves in the solvent to give a yellow or yellowish green solution and leaves a colourless or very pale yellow supernatant aqueous layer.) Add 5 *N* hydrochloric acid in excess; the still warm upper layer becomes red or pink if tin is present. (If only traces of tin are present, the liquid should be set aside for a few minutes.)

Applications to elementary qualitative analysis

The results given earlier in this paper and in other papers[1,3,4,8] would appear to be of value in elementary analysis.

The dropwise addition of dithiol (*a*) to a strongly acid test solution, followed by dilution, and (*b*) to a test solution mixed with pyridine in excess, at once throw light on cations likely to be present or absent, and may identify several cations in less than 1 minute.

In group 1, after the usual separations have been carried out, the addition of a trace of zinc dithiol followed by warming the solution immediately confirms lead. Silver is confirmed by dissolving silver chloride in concentrated hydrochloric acid, adding zinc dithiol, warming the solution and adding water dropwise. At a certain point in the dilution the bright yellow silver complex forms suddenly. Zinc dithiol may also be used to confirm mercury (see p. 400).

In group 2A the procedure described on p. 397 may be used directly on a solution containing mercury, copper, bismuth, lead and cadmium—only cadmium, detected by the addition of dithionite to an ammoniacal solution, followed by passage of hydrogen sulphide,[16] fails to give a characteristic precipitate.

Table I

Analysis of mixtures containing mercury^{II}, copper^{II}, bismuth, lead and cadmium by method of consecutive precipitation with dithiol

The procedure used was that described on p. 397

Volume of solution used, ml	Concentration of cations in solution					
	Mercury^{II}, μg per ml	Copper^{II}, μg per ml	Bismuth, μg per ml	Lead, μg per ml	Cadmium, μg per ml	
2·0	80	1000	1670	1660	885	Hot solution, 4 to 5 *N* in hydrochloric acid. Mercury precipitate just obvious. Might be confused with dithioldisulphide, but suspension of precipitate in ethylene dichloride darkened on passing hydrogen sulphide.
2·0	1000	6	1045	0	0	Acidity reduced to 2 to 2·5 *N*. Solution hot. Black copper precipitate obvious, brownish yellow colour in ethylene dichloride. Solvent shaken with ammonia solution and zinc dithiol gave intense orange aqueous layers.
2·0	1600	50	1670	1660	885	
2·0	495	155	26	510	275	Solution cooled and dithiol addition continued. Intense red bismuth reactions obtained.
2·0	1600	1000	84	1660	885	
1·0	200	63	209	14	0	Sodium acetate crystals added in excess. Solution heated to keep a trace of bismuth complex in solution. Strong yellow lead reactions obtained.
2·0	1600	1000	1670	83	885	

If the more usual method of separation is used, mercury may be confirmed as in group 1; lead by treating lead sulphate with sodium hydroxide and adding zinc dithiol, the precipitate at once becoming bright yellow; bismuth by adding zinc dithiol to the warm weakly acid solution of bismuth hydroxide in dilute hydrochloric acid and cooling, a dark red precipitate being formed; copper by adding zinc dithiol to a drop of the ammoniacal solution after removal of bismuth, the solution becoming orange.[3]

In group 2B, arsenic is immediately confirmed with zinc dithiol.[1] If antimony is separated by the use of zinc dust in 5 *N* hydrochloric acid and re-dissolved as antimony^{V}, a drop of the solution treated with pyridine and dithiol becomes red.[3] Addition of zinc dithiol to the filtrate from the zinc reduction gives the red tin complex.[8]

In groups 3 and 4, zinc dithiol in pyridine affords a sensitive reagent for cobalt and iron and may be of use for nickel.[3] Diacetyldithiol may be used to confirm manganese[3] and quinoxaline-2:3-dithiol affords a sensitive test for nickel.[17] Prior dissolution of cobalt and nickel sulphides is unnecessary.[17]

Most of the reactions mentioned are sensitive and selective. It would appear that dithiol, especially in the form of its zinc complex, affords a useful multi-purpose reagent. As only minute amounts are required for confirmatory tests, the cost is low. As a general laboratory reagent, a suspension in ethanol has been found to be satisfactory. It can be prepared as follows—

> Rub 1 g of zinc dithiol with ethanol in a mortar and pour off the suspension. Repeat until all lumps have been broken up. Dilute to 100 ml with ethanol and store in a bottle fitted with dropping tube and teat, and mark the bottle "Shake before use."
>
> For most tests, 1 to 5 drops are adequate.

I express my thanks to Professor H. J. Emeléus, Mr. P. S. Jewell and Dr. F. G. Mann for their interest and to Hopkin and Williams Ltd. for a gift of zinc dithiol and diacetyldithiol.

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NOTE—References 1, 3, 4, 8 and 14 are to Parts V, III, IV, I and VI, respectively, of this series.

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The Use of Alkaline Reagents to Determine Carbohydrate Reducing Groups

Part I. 3:5-Dinitrosalicylate Ion, and Interference by Air

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The determination of glucose and maltose with the 3:5-dinitrosalicylate reagent of Sumner has been carried out at different concentrations of alkali and in the presence and absence of air. It is shown that the removal of air straightens the calibration line and greatly increases the sensitivity of the reagent at low concentrations of sugar.

ALTHOUGH many carbohydrates are unstable in alkaline solution, especially if air is present, many semi-micro methods of determining reducing sugars are carried out under hot alkaline conditions without the exclusion of air. It is therefore pertinent to determine the optimum degree of alkalinity, and the extent of interference by air, for these methods.

In view of the occasional use of these methods for the analysis of the reducing end-groups of polysaccharides, a general remark must first be made. During the determination of simple reducing sugars, it may be possible to make an accurate allowance for the side effects of alkali and oxygen by calibration under equivalent conditions. When, however, it is desired to determine the reducing groups of a polysaccharide, there may be no feasible method of calibration if, simultaneously with the main reaction, oxidative degradation with scission at random points along the carbohydrate chain produces new reducing groups.

This paper deals with the influence of oxygen and the concentration of alkali on the reaction of the 3:5-dinitrosalicylate ion with glucose and maltose. 3:5-Dinitrosalicylate ion was introduced by Sumner,[1,2] to replace the picric acid reagent of Lewis and Benedict[3] for the semi-micro determination of reducing sugars, and its use depends on the colorimetric determination of the reddish brown reduction product formed when the yellow alkaline solution of 3:5-dinitrosalicylate ion is heated with sugars. The method has found a number of applications, for instance, in the determination of blood sugar,[4] the reducing end-groups of the components of starch[5,6,7] and diastatic activity.[8]

Sumner[2] recognised that alkali and oxygen compete with 3:5-dinitrosalicylate ion, and, in conformity with the work of Benedict,[9] showed that glucose loses its reducing power if heated for a few minutes beforehand in alkali in the presence of air. He therefore added 25 per cent. w/v of Rochelle salt to reduce the concentration of oxygen in the solution before heating. Sumner and Sisler[4] also found it necessary to remove oxygen from blood by a stream of nitrogen before determining blood sugar, but they did not carry out the actual reaction with 3:5-dinitrosalicylate ion under nitrogen.

A variety of conditions have been described for the reaction. Sumner[2] selected 0·25 *M* sodium hydroxide and a temperature of 100° C, conditions that were also used by Hostettler, Borel and Deuel.[10] Others have used 0·167 *M* sodium hydroxide at 100° C,[8] 0·5 *M* sodium hydroxide at 100° C,[4] 1·5 *M* sodium hydroxide at 65° C[5] and 2 *M* sodium hydroxide at 65° C.[6]

To assess the effect of oxygen, maltose was determined under representative conditions first in the presence of air, and then after air had been removed by a stream of nitrogen. Experiments were then carried out in which the concentration of alkali was varied.

Experimental

Apparatus—

Reactions were carried out in 14-cm × 2-cm Pyrex-glass tubes, all of similar thickness. Determinations in air, and in the absence of air, were made in the same tubes, but when air was removed the tubes, instead of being left open, were fitted through B19 joints with Drechsel-type bubblers. These were attached to a nitrogen train by B10 sockets. The nitrogen out-flow was passed through a small glass water-trap attached by a B10 socket.

Rubber connections were rigorously excluded, as they had been found to give rise to high reagent blank values. Oxygen-free nitrogen was passed for 20 minutes through the reaction mixtures before heating.

REAGENTS—

Potassium hydroxide solution, 6 M.

3:5-*Dinitrosalicylate reagent solution*—A 1·5 per cent. w/v solution of 3:5-dinitrosalicylic acid (obtained from the British Drug Houses Ltd.) in 0·2 *M* potassium hydroxide.

3:5-*Dinitrosalicylate - Rochelle salt reagent solution*—A solution of 0·5 g of 3:5-dinitrosalicylic acid and 25·5 g of Rochelle salt in 41·3 ml of *M* potassium hydroxide, diluted to 100 ml with water.

Standard glucose solution.

Standard maltose solution.

Commercial 3:5-dinitrosalicylic acid was used without further purification, since even after 30 minutes in 0·28 *M* potassium hydroxide at 100° C the reagent blank solutions reached an optical density of only 0·003 (contrast Meyer, van der Wyk and Feng[11]). On the other hand, it was found that some samples of alkali gave rise to reagent blank solutions that increased rapidly in optical density during heating. These samples of alkali were rejected.

REACTION MIXTURES—

Three reaction mixtures were used, as follows—

(*a*) 1 ml of 3:5-dinitrosalicylate reagent solution, 1 ml of 6 *M* potassium hydroxide and 2 ml of sugar solution;

(*b*) 1 ml of 3:5-dinitrosalicylate reagent solution, 0·2 ml of 6 *M* potassium hydroxide and 3·8 ml of sugar solution;

(*c*) 3·75 ml of 3:5-dinitrosalicylate - Rochelle salt reagent solution and 1·25 ml of sugar solution.

PROCEDURE—

The reaction mixtures, with or without de-aeration as required, were heated for 30 minutes in a water bath kept at 65° ± 0·1° C, or for 5 minutes in a boiling-water bath (see Figs. 1, 2 and 3). After they had been heated, the tubes were quickly cooled to room temperature and their contents diluted to 25 ml with water. The optical densities of the solutions were measured in 4-cm cells with a Spekker absorptiometer at 520 mμ against a reagent blank solution kept at the same temperature.

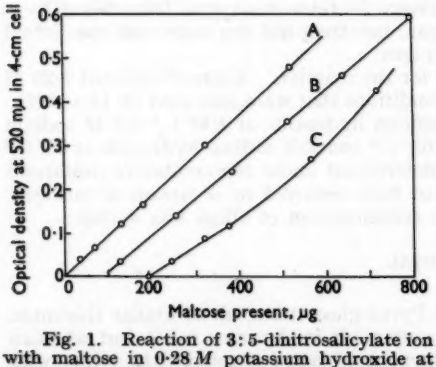

Fig. 1. Reaction of 3:5-dinitrosalicylate ion with maltose in 0·28 *M* potassium hydroxide at 65° C for 30 minutes: curve A, in nitrogen; curve B, in air with Rochelle salt added to the reaction mixture; curve C, in air

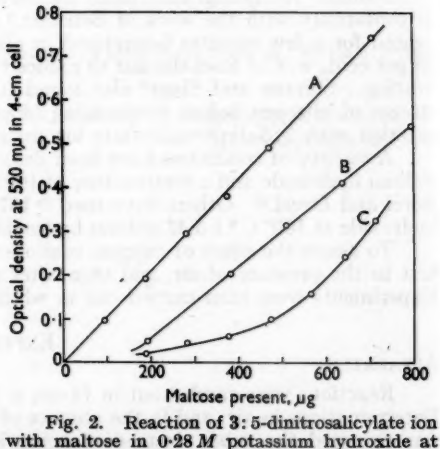

Fig. 2. Reaction of 3:5-dinitrosalicylate ion with maltose in 0·28 *M* potassium hydroxide at 100° C for 5 minutes: curve A, in nitrogen; curve B, in air with Rochelle salt added to the reaction mixture; curve C, in air

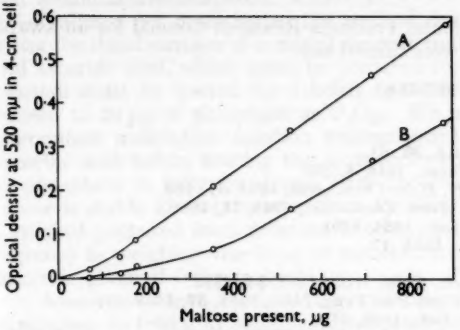

Fig. 3. Reaction of 3:5-dinitrosalicylate ion with maltose in 1·55 *M* potassium hydroxide at 65° C for 30 minutes: curve A, in nitrogen; curve B, in air

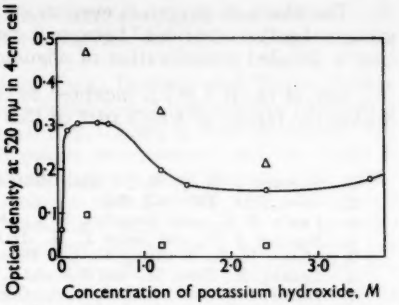

Fig. 4. Effect of the concentration of alkali on the reaction of 3:5-dinitrosalicylate ion with sugars in an atmosphere of nitrogen: ○, 0·29 mg of maltose at 65° C for 30 minutes; △, 0·33 mg of glucose at 65° C for 30 minutes; □, 0·33 mg of glucose at 65° C for 30 minutes, but first heated with the alkali alone in an atmosphere of nitrogen at 65° C for 30 minutes

Control of the temperature during the measurement of optical density is necessary because of the thermochromism of the 3:5-dinitrosalicylate ion.[12]

In addition to the determinations recorded in Figs. 1, 2 and 3, a series of determinations was made with a fixed sugar concentration, but at different concentrations of alkali (see Fig. 4). Also in Fig. 4 are recorded the results of determinations in which glucose had been heated with alkali under nitrogen for 30 minutes before the addition of the 3:5-dinitrosalicylate reagent solution.

STANDARD DEVIATION IN THE DETERMINATION OF GLUCOSE—

From a consideration of the experimental results in Figs. 1, 2, 3 and 4, it was decided to remove oxygen, but otherwise to adopt conditions near to those used by Sumner,[2] *viz.*, 0·28 *M* potassium hydroxide and 5 minutes' heating at 100° C. Reaction mixture (*b*) was used.

Under these conditions the standard deviation of the increase in optical density produced by 135 μg of glucose was determined, the results being as follows—

Tube No. ..	1	2	3	4	5	6	7	8	9	10
Optical density	0·148	0·151	0·152	0·147	0·154	0·152	0·151	0·151	0·150	0·151

Mean increase in optical density = 0·151 ± 0·002.
Standard deviation = 1·4 per cent.

DISCUSSION OF RESULTS

From the results shown in Figs. 1, 2 and 3 for the reduction of 3:5-dinitrosalicylate ion by maltose, it can be seen that the removal of oxygen produces a significant increase in sensitivity and in linearity of the plot of optical density against concentration of sugar under the conditions of temperature and alkalinity in common use. Since the oxygen content of a hot solution exposed to the air is not an easily controlled quantity, it may also be expected that the reproducibility will be improved by the exclusion of air.

Besides the preventable destruction of sugar by oxygen, alkali itself[2,9,13] is competing with 3:5-dinitrosalicylate ion throughout the reaction. This is shown (see Fig. 4) by the decreased formation of colour at higher alkalinity and by the experiments in which glucose was pre-heated in alkali alone (in the absence of air) before determination with 3:5-dinitrosalicylate ion. It is therefore advisable to keep the concentration of alkali as low as is consistent with a rapid reaction rate. The results suggest that about 0·3 *M* alkali is optimal, as proposed by Sumner.[2] The more concentrated alkali introduced by Meyer, Noelting and Bernfeld[5] seems to serve no useful purpose and lowers the sensitivity of the reagent (see Fig. 3). In our experimental work it also led to non-linearity of the calibration curve for maltose, even when determinations were carried out under nitrogen.

Rochelle salt removes some of the interfering oxygen, but still leaves enough to destroy about 0·1 mg of maltose, according to the intercepts in Figs. 1 and 2, and in Fig. 2 in Waldt's paper.[8]

The effect of oxygen is even more important in the determination of the reducing end-groups of polysaccharides,[7] because it causes scission of the polysaccharide chain. It is hoped that a detailed consideration of oligosaccharides and polysaccharides will be published later.

One of us (R.T.B.) is indebted to the Colonial Products Research Council for an award, during the tenure of which part of this work was carried out.

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The Colorimetric Determination of Phosphorus

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In the colorimetric determination of phosphate by Denigès's method the molybdophosphate is reduced by the addition of stannous chloride. In the method described, stannous chloride has been replaced by ascorbic acid. Colour development is rapid at the boiling-point of the solution, and, once developed, the molybdenum blue colour is extremely stable at room temperature. The stability of the molybdenum blue allows the colour intensity to be measured either visually or instrumentally. Instrumental measurement has made it possible to apply the method to phosphate concentrations in the range 1 to 600 μg (as P_2O_5).

The ascorbic acid is added in solid form, since its solution is unstable. Reactions with arsenic and silica have been investigated, and application of the method to the determination of phosphate in boiler water and effluents is described.

As a result of our work on the determination of selenium,[1] it occurred to us that ascorbic acid might be a suitable reagent to replace stannous chloride for the reduction of molybdophosphate in the colorimetric determination of phosphate by Denigès's method.[2]

Holman and Pollard[3] have modified Denigès's method and use a colour disc to match the colours in a Lovibond Nessleriser. Interfering elements have been investigated by the previously mentioned workers, and it is of significant importance to us that ferric iron must not be present in amounts greater than 1 p.p.m. in the final solution. Although this may cause no concern in water analysis, since most waters contain less than this amount of iron, it will undoubtedly be of significance in the analysis of effluents. Another point of importance is that the stannous chloride solution must be freshly prepared, but it is doubtful whether each batch of solution prepared contains exactly the same concentration of the stannous salt.

In our experience, different calibration curves are obtained with standard concentrations of phosphate when ammonium molybdate from different sources is used in Holman and Pollard's[3] modification of Denigès's method.

In a literature survey we found that Ammon and Hinsberg[4] were the first to use ascorbic acid for the reduction of molybdophosphate. Lowry, Roberts, Leiner, Wu and Farr[5] later modified the procedure of Ammon and Hinsberg by using a more concentrated solution of ascorbic acid and heating for a longer time at 37° C. Chen, Toribara and Warner[6] have applied the method modified by Lowry, Roberts, Leiner, Wu and Farr to the determination

of phosphorus in blood, plasma, serum and urine, and have compared the method with that of Fiske and Subbarow,[7] in which the reduction is carried out with sodium sulphite and aminonaphtholsulphonic acid.

We have not investigated the method described by Chen, Toribara and Warner,[6] since it has the disadvantage of a mixed reagent containing sulphuric acid, ammonium molybdate and ascorbic acid, which must be prepared freshly each day. Also, in their procedure, the solution must be heated for 2 hours to achieve full colour development and the range is limited to 20 μg of phosphate as P_2O_5. We have found that, by using the sulphuric acid - ammonium molybdate solution recommended by Holman and Pollard[3] and adding solid ascorbic acid before heating the solution to boiling, the blue colour formed in the presence of phosphate is fully developed after the solution has been boiled for 1 minute and the colour is stable at room temperature for several weeks. The colours are reproducible with a reagent prepared from different batches of ammonium molybdate. No great accuracy is required in weighing the 0·1 g of ascorbic acid used in each determination, since the same result is given if 0·2 g is used.

Amounts of iron up to 0·02 g, chloride equivalent to 2 g of sodium chloride, nitrate equivalent to 0·05 g of sodium nitrate and sulphate and perchlorate equivalent to at least 5 g of sodium sulphate and 5 g of sodium perchlorate have no effect on the proposed method. The presence of 0·05 g of soluble silica can also be tolerated.

Arsenic present as arsenate gives the same blue colour as phosphate, but, if the arsenate is reduced to arsenite, 0·01 g of arsenic has no effect. We have reduced the arsenate with sodium metabisulphite, using a volume of sulphuric acid equivalent to the weight of meta-bisulphite used. It was necessary to set the solution aside for 4 hours in order to reduce the arsenate completely.

Organic matter can be destroyed by wet oxidation, and, if a sample contains sodium chloride or sodium nitrate in amounts greater than those mentioned, chloride and nitrate can be removed by evaporation with a slight excess of sulphuric acid. The solution is then neutralised before the method is applied.

Meta- and pyrophosphates are partly hydrolysed during the method and should therefore be completely hydrolysed before application of the method to a sample containing these phosphates.

Experimental

The reagents used for the experimental work are described on p. 413.

In our first series of experiments, 0, 5·0, 10·0, 15·0, 20·0 and 25·0-ml portions of the dilute phosphate solution were placed in six 100-ml beakers and each solution was diluted to 40 ml with distilled water. One millilitre of ammonium molybdate - sulphuric acid solution was added to each, and then 0·1 g of ascorbic acid, after which the solutions were stirred until the ascorbic acid had dissolved. No colour developed after the solutions had been standing for several minutes. Each solution was heated to the boiling-point, when it appeared that the ascorbic acid had reduced the molybdophosphate completely. Boiling was continued for 1 minute. The solutions were cooled, transferred to 50-ml calibrated flasks and then each was diluted to the mark. The optical density of each solution was measured in a 1-cm cell with a Spekker absorptiometer, Ilford No. 608 red filters being used. Distilled water was used in the comparison cell. The results, corrected for a blank value of 0·007, were as follows—

Amount of phosphate, as P_2O_5, μg ..	25	50	100	150	200	250
Corrected indicator-drum reading.. ..	0·065	0·130	0·265	0·398	0·528	0·660

It can be seen that, when the weight of phosphate is plotted against the corrected indicator-drum reading, the graph is linear.

A further series of tests with amounts of phosphate between 0 and 50 μg was carried out, the final colour measurement being made in 4-cm cells. The results, corrected for a blank value of 0·017, were as follows—

Amount of phosphate, as P_2O_5, μg ..	5	10	20	30	40	50
Corrected indicator-drum reading.. ..	0·053	0·108	0·215	0·325	0·428	0·533

Effect of higher concentration of ammonium molybdate - sulphuric acid solution—

Two series of experiments were carried out. In six 100-ml beakers, 0, 5·0, 10·0, 15·0, 20·0 and 25·0-ml portions of the dilute phosphate solution were placed and each was diluted

to 40 ml. Three millilitres of the ammonium molybdate - sulphuric acid solution were added to each and then 0·1 g of ascorbic acid. The solutions were stirred until the ascorbic acid had dissolved. Each solution was heated to the boiling-point and boiled for 1 minute.

The solutions were cooled and each was transferred to a 50-ml calibrated flask and then diluted to the mark. The optical densities of the solutions were identical with those for the first series of results shown above.

A similar series of tests was carried out in which 5·0 ml of the ammonium molybdate - sulphuric acid solution were added. The results were again identical with those for the first series shown above.

REACTION WITH SILICA—

It is well known that, under certain conditions of acidity and concentration of molybdate, silica will produce a yellow molybdosilicate complex, which can be reduced to form a molybdenum blue - silica complex. It was therefore important to know how silica behaved under the conditions of the method.

Since the samples we were likely to analyse for phosphate would contain a relatively high proportion of silica, we investigated the effect of silica in fairly high concentration.

A solution of sodium silicate was prepared, 1 ml of which contained 0·0025 g of silica. In six 100-ml beakers, 0, 4·0, 8·0, 12·0, 16·0 and 20·0-ml portions of this solution were placed. Each solution was diluted to 40 ml and then treated further with 1 ml of ammonium molybdate - sulphuric acid solution and 0·1 g of ascorbic acid as described for the phosphate test. The optical densities were measured in 1-cm cells; the results, corrected for a blank value of 0·005, were as follows—

Amount of silicate, as SiO_2, g	0·01	0·02	0·03	0·04	0·05
Corrected indicator-drum reading	0·060	0·070	0·105	0·125	0·155

It can be seen from these results that silica reacts under the conditions of the method, but the relationship between increasing silica content and indicator-drum reading is not linear.

We have shown that different amounts of ammonium molybdate - sulphuric acid solution produce the same colour intensity with equal amounts of phosphate. We decided, therefore, to ascertain the effect of different amounts of ammonium molybdate - sulphuric acid solution on its reaction with silicate.

A series of tests was made with the solution of sodium silicate. Portions of the solution each containing 0·05 g of silica were measured into five beakers, each solution was diluted to 40 ml and different volumes of the ammonium molybdate - sulphuric acid solution and 0·1 g of ascorbic acid were added to each. The solutions were heated to the boiling-point and boiled for 1 minute, after which they were cooled, transferred to 50-ml calibrated flasks and diluted to the mark. The optical densities were measured in 1-cm cells; the results were as follows—

Amount of ammonium molybdate - sulphuric acid solution added, ml	1·0	2·0	3·0	4·0	5·0
Indicator-drum reading	0·150	0·060	0·025	0·010	0·010

The method was then applied to solutions containing both silicate and phosphate, 4 ml of the ammonium molybdate - sulphuric acid solution being used. The results are shown in Table I.

TABLE I

OPTICAL DENSITY OF SOLUTIONS CONTAINING PHOSPHATE AND SILICATE

The optical densities were measured in 1-cm cells

Amount of phosphate present, as P_2O_5, μg	Amount of silicate added, as SiO_2, g	Indicator-drum reading	Indicator-drum reading, corrected for blank value
0	0·05	0·015	—
0	0·03	0·012	—
0	0·02	0·012	—
0	0·01	0·010	—
10	0·03	0·040	0·028
20	0·05	0·070	0·055
50	0·05	0·150	0·135
100	0·02	0·278	0·266
150	0·01	0·405	0·395
250	0·05	0·675	0·660

By comparing the results in Table I with those obtained with phosphate alone in presence of 4 ml of ammonium molybdate - sulphuric acid solution, it can be seen that interference from between 0·01 and 0·05 g of silicate, as SiO_2, is negligible. The optical densities of the solutions of phosphate alone were measured in 1-cm cells; the results, corrected for a blank value of 0·007, were as follows—

Amount of phosphate, as P_2O_5, μg	10	20	30	40	50	100	150	250
Corrected indicator-drum reading	0·025	0·048	0·073	0·100	0·130	0·265	0·398	0·660

Since we had now established the volume of ammonium molybdate - sulphuric acid solution that was suitable for the determination of phosphate without interference from silicate, all subsequent experiments were carried out with 4 ml of this reagent.

EFFECT OF INCREASE IN AMOUNT OF ASCORBIC ACID—

A series of experiments was carried out exactly as described under "Method" on p. 413, except that different amounts of ascorbic acid were used. The results are shown in Table II.

TABLE II

EFFECT OF ASCORBIC ACID ON OPTICAL DENSITY OF PHOSPHATE SOLUTIONS

The optical densities were measured in 1-cm cells

Amount of phosphate present, as P_2O_5, μg	Indicator-drum reading	Indicator-drum reading, corrected for blank value	Colour of final solution
In presence of 0·2 g of ascorbic acid—			
0	0·005	—	—
10	0·033	0·028	Blue
20	0·060	0·055	Blue
50	0·135	0·130	Blue
100	0·270	0·265	Blue
150	0·403	0·398	Blue
200	0·533	0·528	Blue
250	0·665	0·660	Blue
In presence of 0·5 g of ascorbic acid—			
0	0·010	—	Yellow
10	0·040	0·030	Yellowish green
20	0·065	0·055	Yellowish green
50	0·140	0·130	Green
100	0·276	0·266	Blue-green
150	0·408	0·398	Blue
200	0·536	0·526	Blue
250	0·668	0·658	Blue

Although the indicator-drum readings with these higher amounts of ascorbic acid agree with those found when 0·1 g is used, the colours obtained with 0·5 g of ascorbic acid were less satisfactory visually.

Since excellent colours and results were obtained with 0·1 g of ascorbic acid, we adhered to this amount in all subsequent work. The ascorbic acid was added in solid form and not as a solution, since the latter slowly deteriorates.

STABILITY OF THE MOLYBDENUM BLUE COLOUR—

A series of standards was prepared as described under "Preparation of Calibration Curves" on p. 413. The optical density of each solution was measured immediately in 1-cm cells; the results were as follows—

Amount of phosphate, as P_2O_5, μg	0	10	50	100	250
Indicator-drum reading	0·007	0·033	0·137	0·272	0·667

These solutions were again measured after 1 day, 1 week, 1 month and 3 months. Over this period there was no change in the optical density of any of the solutions.

RANGE OF THE METHOD—

The method can be modified to cover a wider range of phosphate concentration than shown previously by diluting the final solution to 100 ml and using 1-cm cells in the measurement of optical density. Typical results, corrected for a blank value of 0·005, were as follows—

Amount of phosphate, as P_2O_5, μg	250	300	400	500	600
Corrected indicator-drum reading	0·335	0·405	0·540	0·675	0·810

Similarly, the method can be made to cover a very low range of phosphate concentration by diluting the final solution to 50 ml and measuring the optical density in 4-cm cells or larger.

EFFECT OF SODIUM CHLORIDE—

Solutions were prepared each containing 2 g of sodium chloride dissolved in 10 ml of distilled water. Different amounts of phosphate were added to each and the method was applied. The results obtained were exactly the same as when phosphate alone was present.

A similar series of experiments was then carried out in the presence of 3 g of sodium chloride, the results of which are shown in Table III.

TABLE III

EFFECT OF PRESENCE OF 3 g OF SODIUM CHLORIDE ON RECOVERY OF PHOSPHATE

The optical densities were measured in 1-cm cells

Amount of phosphate added, as P_2O_5, μg	Indicator-drum reading	Indicator-drum reading, corrected for blank value	Amount of phosphate found, as P_2O_5, μg
0	0·005	—	—
10	0·030	0·025	10
50	0·120	0·115	43
100	0·237	0·232	87
150	0·327	0·322	122
200	0·465	0·460	172
250	0·530	0·525	198

EFFECT OF SODIUM SULPHATE—

Several solutions were prepared containing 2, 3 and 5 g of sodium sulphate and different amounts of phosphate, and the method was applied as described. The results were exactly the same as when phosphate alone was present.

EFFECT OF SODIUM NITRATE—

When the method was applied to solutions containing 1 g of sodium nitrate and different amounts of phosphate, no blue colour was formed. Further experiments showed that only 0·05 g of added sodium nitrate could be tolerated. The molybdenum blue colour was not stable and faded within 12 hours.

Since sodium chloride in amounts above 2 g per 50 ml of final solution and sodium nitrate above 0·05 g interfere with the method, but sodium sulphate up to 5 g has no effect, it is obvious that interference from chloride and nitrate can be overcome by evaporation with sulphuric acid and subsequent neutralisation of the acid with sodium hydroxide. Similarly, organic matter interferes with the test, but can be destroyed with nitric and sulphuric acids, the test being applied on the resulting solution after neutralisation.

EFFECT OF SODIUM PERCHLORATE—

Since the destruction of organic matter in a sample is often accomplished more effectively by using perchloric acid as well as nitric and sulphuric acids, the effect of sodium perchlorate on the method was investigated.

Several solutions were prepared containing 1 and 5 g of sodium perchlorate and different amounts of phosphate. The method was applied and the results were exactly the same as when phosphate alone was present.

EFFECT OF FERRIC IRON—

When the method was applied to solutions containing known amounts of phosphate and 0·02 g of iron, added as ferric chloride, excellent results were obtained, but with increased amounts of iron the results were low. In the presence of 0·03 g of iron, the recoveries of added phosphate varied from approximately 60 to 80 per cent. with increasing amounts of phosphate.

EFFECT OF ARSENIC—

Under the conditions of the method, arsenate gives the molybdenum blue reaction. In a series of experiments we found that, if the arsenate were first reduced to arsenite, relatively large amounts of arsenic gave no reaction.

Solutions were prepared containing different amounts of phosphate and to each was added 0·01 g of arsenic as sodium arsenate. The solutions were diluted to 30 ml and to each was added 1 g of sodium metabisulphite and then 12 ml of *N* sulphuric acid. The solutions were set aside for 4 hours, after which 4·0 ml of ammonium molybdate - sulphuric acid solution were added and then 0·1 g of ascorbic acid. The solutions were heated to boiling-point and boiled for 1 minute. They were then cooled, diluted to the mark in 50-ml calibrated flasks and the optical density of each was determined. The results are shown in Table IV.

TABLE IV

EFFECT OF ARSENIC ON THE RECOVERY OF PHOSPHATE

Each sample contained 0·01 g of arsenic, as sodium arsenate
The optical densities were measured in 1-cm cells

Amount of phosphate added, as P_2O_5, μg	Indicator-drum reading	Indicator-drum reading, corrected for blank value	Amount of phosphate found, as P_2O_5, μg
0	0·015	—	—
10	0·040	0·025	10
50	0·145	0·130	49
100	0·277	0·262	99
150	0·410	0·395	149
200	0·545	0·530	200
250	0·675	0·660	250

APPLICATION TO SOLUTIONS CONTAINING CHLORIDE, NITRATE AND ORGANIC MATTER—

Determinations of phosphate were carried out on solutions containing sodium chloride, sodium nitrate, sucrose and phosphate. Organic matter was destroyed and the determination carried out exactly as described under "Determination of Phosphate in Effluents" on p. 414. The results are shown in Table V.

TABLE V

RECOVERY OF PHOSPHATE FROM SOLUTIONS CONTAINING CHLORIDE, NITRATE AND ORGANIC MATTER

Each sample contained 0·2 g of sucrose, 3 g of sodium nitrate and 2 g of sodium chloride
The optical densities were measured in 1-cm cells

Amount of phosphate added, as P_2O_5, μg	Indicator-drum reading	Indicator-drum reading, corrected for blank value	Amount of phosphate found, as P_2O_5, μg
0	0·020	—	—
20	0·075	0·055	21
50	0·150	0·130	50
100	0·285	0·265	100

SENSITIVITY OF THE METHOD—

Comparison of the sensitivity of the method with Holman and Pollard's modification of Denigès's method was made, and, as the results in Table VI show, the sensitivity is about half that of Denigès's method.

TABLE VI

SENSITIVITY OF THE METHOD

The optical densities were measured in 4-cm cells

Amount of phosphate added, as P_2O_5, μg	Indicator-drum reading when Holman and Pollard's method was used	Indicator-drum reading when the proposed method was used
5	0·130	0·053
10	0·258	0·108
15	0·388	0·160
20	0·515	0·215
25	0·635	0·270

We are of the opinion, however, that this reduction in sensitivity is well compensated for by the wide range of phosphate concentration that the method will cover, its relative freedom from interference and the stability of the molybdenum blue colour.

APPLICATION TO THE DETERMINATION OF PHOSPHATE IN BOILER WATER—

A sample of boiler water with the following composition was used for the experiments.

Sodium carbonate	689 p.p.m.
Sodium hydroxide	2000 p.p.m.
Sodium sulphate	7029 p.p.m.
Silica	37 p.p.m.
Sodium sulphite	143 p.p.m.
Sodium chloride	1958 p.p.m.

Five-millilitre portions of the boiler water were measured into 100-ml beakers and sufficient *N* sulphuric acid was added to neutralise the alkalinity. The amount of *N* sulphuric acid required was determined on a separate portion of the boiler water. Known volumes of standard phosphate solution were added to the neutralised solutions, each solution was diluted to 40 ml and the phosphate was determined as described under "Procedure," beginning at "Add 4·0 ml of the ammonium molybdate - sulphuric acid solution." The results are shown in Table VII.

TABLE VII

RECOVERY OF PHOSPHATE FROM BOILER WATER

Amount of phosphate added, as P_2O_5, μg	Indicator-drum reading (1-cm cell)	Indicator-drum reading (4-cm cell)	Total amount of phosphate found, as P_2O_5, μg	Corrected amount of phosphate found, as P_2O_5, μg
0	0·008	0·032	3	—
20	—	0·247	23	20
50	—	0·555	52	49
100	0·280	—	105	102
200	0·542	—	205	202
500*	0·680	—	503	500

* Final solution diluted to 100 ml.

APPLICATION TO THE DETERMINATION OF PHOSPHATE IN EFFLUENTS—

A sample of effluent with the following composition was used for the experiments.

Calcium bicarbonate	235 p.p.m.
Calcium sulphate	196 p.p.m.
Calcium chloride	610 p.p.m.
Sodium chloride	1400 p.p.m.
Magnesium chloride	66 p.p.m.
Organic matter	100 p.p.m.

To a series of 50-ml portions of the effluent, known volumes of standard phosphate solution were added and the determination was carried out as described under "Determination of Phosphate in Effluents." The results are shown in Table VIII.

TABLE VIII

RECOVERY OF PHOSPHATE FROM EFFLUENT

Amount of phosphate added, as P_2O_5, μg	Indicator-drum reading (1-cm cell)	Indicator-drum reading (4-cm cell)	Total amount of phosphate found, as P_2O_5, μg	Corrected amount of phosphate found, as P_2O_5, μg
0	0·025	0·100	9·5	—
10	—	0·218	20	10·5
20	—	0·320	29·5	20
50	0·160	—	60	50·5
100	0·300	—	113	103·5
200	0·563	—	212·5	203
500*	0·690	—	512	502·5

* Final solution diluted to 100 ml.

It can be seen that, in addition to the known composition, the effluent contained a small amount of phosphorus.

Method

Reagents—

Ammonium molybdate - sulphuric acid solution—Dissolve 10·0 g of crystalline ammonium molybdate in about 70 ml of distilled water and dilute the solution to 100 ml. Carefully add 150 ml of sulphuric acid, sp.gr. 1·84, to 150 ml of distilled water, mixing the solution during the addition. Cool the solution, add the ammonium molybdate solution carefully and with mixing to the diluted sulphuric acid and allow the mixture to cool.

Ascorbic acid.

Standard phosphate solution—Dissolve 0·7669 g of potassium dihydrogen orthophosphate in distilled water and dilute the solution to 1 litre. For use, dilute 25 ml of this solution to 1 litre.

$$1 \text{ ml} \equiv 10 \ \mu\text{g of } P_2O_5.$$

Procedure—

Measure a suitable volume of the sample solution containing the phosphate present as orthophosphate, neutralise it and adjust the volume to 40 ml either by dilution or evaporation. Add 4·0 ml of the ammonium molybdate - sulphuric acid solution and mix. Add 0·1 g of ascorbic acid, heat the solution to boiling-point and boil for 1 minute. Cool the solution, transfer it to a 50-ml calibrated flask (or a 100-ml calibrated flask for phosphate contents between 250 and 600 μg) and dilute to the mark with distilled water.

Carry out a blank test on the reagents with distilled water in place of the sample.

Measure the optical densities of the blank and test solutions in either 1-cm or 4-cm cells in an absorptiometer with Ilford No. 608 red filters. From a previously prepared calibration curve, read the amounts of phosphate present in the two solutions.

Preparation of calibration curves—

For the range 0 to 50 μg of phosphate—In seven 100-ml beakers place 0·0, 0·5, 1·0, 2·0, 3·0, 4·0 and 5·0-ml portions of standard phosphate solution. Dilute each solution to 40 ml and continue as described under "Procedure." Measure the optical densities in 4-cm cells.

For the range 0 to 250 μg of phosphate—In a series of beakers place 0·0, 5·0, 7·5, 10·0, 12·5, 15·0, 17·5, 20·0, 22·5 and 25·0-ml portions of standard phosphate solution. Dilute each solution to 40 ml and continue as described under "Procedure." Measure the optical densities in 1-cm cells.

For the range 250 to 600 μg of phosphate—In a series of beakers place 0·0 and from 25·0 to 60·0-ml portions, in steps of 5 ml, of standard phosphate solution. Adjust the volumes of the solutions to 40 ml either by dilution or evaporation and continue as described under "Procedure," but dilute the final solutions to 100 ml. Measure the optical densities in 1-cm cells.

Applications of the method

Determination of phosphate in boiler water—

Calgon is frequently used in the treatment of boiler-feed water to prevent the formation of scale and it is often required to know the phosphate content of the boiler blow-down water. During the time the water is in the boiler, the Calgon is usually completely hydrolysed to orthophosphate. Direct application of the method can therefore be carried out on a suitable volume of the sample that has been neutralised with *N* sulphuric acid. The recommended procedure is as follows.

Measure 20 ml of the sample into a 100-ml calibrated flask. Add sufficient *N* sulphuric acid to neutralise the alkalinity and dilute to the mark. Measure 25 ml of the solution into a 100-ml beaker, dilute to 40 ml and continue as described under "Procedure."

For the determination of phosphate present in boiler-feed water as hexametaphosphate we recommend hydrolysis of the hexametaphosphate by neutralisation of the sample, addition of 1 ml of hydrochloric acid, sp.gr. 1·18, and evaporation of the solution to dryness. Complete hydrolysis of hexametaphosphate is not usually achieved simply by boiling an acidified solution of the sample.

DETERMINATION OF PHOSPHATE IN EFFLUENTS—

Measure a suitable volume, *e.g.*, 50 ml, of the sample into a 250-ml beaker. Neutralise the sample by the addition of either dilute sulphuric acid or dilute sodium hydroxide solution. Add 3 ml of sulphuric acid, sp.gr. 1·84, and evaporate the solution on a sand-bath until white fumes of sulphur trioxide appear. If organic matter is present, remove the beaker from the sand-bath, allow it to cool somewhat, add 1 ml of nitric acid, sp.gr. 1·42, and again heat until fumes of sulphur trioxide appear. Again allow to cool somewhat, add 1 ml of nitric acid, 1 ml of 60 per cent. perchloric acid and heat on a sand-bath until fumes of sulphur trioxide appear; organic matter should then have been destroyed.

Allow the solution to cool, add 5 ml of distilled water and again evaporate until fumes appear. Cool the solution, add 25 ml of distilled water, boil for 5 minutes and then neutralise by adding 2·5 *N* sodium hydroxide. Dilute the solution to 40 ml and continue as described under "Procedure," beginning at the addition of 4·0 ml of the ammonium molybdate - sulphuric acid solution.

Carry out a blank test on the reagents.

DETERMINATION OF PHOSPHATE IN SALT DEPOSITS

Take a suitable weight, not exceeding 5 g, of the sample and place it in a 250-ml beaker. Add 15 ml of distilled water and carefully add 7 ml of sulphuric acid, sp.gr. 1·84. Evaporate the solution on a sand-bath until fumes of sulphur trioxide appear. Cool the solution, add 25 ml of distilled water, boil for 5 minutes and then neutralise by adding 2·5 *N* sodium hydroxide. Dilute the solution to 40 ml and continue as described under "Procedure," commencing with the addition of 4·0 ml of the ammonium molybdate - sulphuric acid solution.

Carry out a blank test on the reagents.

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A Difference Photometric Method for Determining Uranium in Perchloric Acid Medium

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A difference spectrophotometric method for determining uranium in perchloric acid medium is described. Factors affecting the accuracy have been studied. It is shown that chromium, vanadium, nickel, cobalt, sulphate and phosphate interfere to a marked degree.

IN difference spectrophotometry, as used in this investigation, a reference solution of relatively high optical density is used in place of the solvent of conventional spectrophotometry and the optical-density scale is set to zero by adjustment of the aperture controlling the amount of light falling on the photomultiplier tube. Slightly more concentrated sample solutions are measured against this reference standard. The optical-density readings are proportional to the difference in concentration between this reference solution and the sample solution, provided that Beer's law applies in the range of concentration.

The theory relating to this principle of measurement has been admirably treated in a number of papers and the optimum conditions for the photometric measurements may now be considered as fairly well established.[1,2,3,4,5] When errors other than those arising from the photometric measurement are neglected, a remarkably high order of precision is attainable. Bacon and Milner,[5] for example, assess the theoretical error as $\pm 0{\cdot}017$ per cent. for uranium

in a sulphuric acid medium; Bastian,[6] who used a slightly different procedure of measurement, obtained a precision of ±0·033 per cent. for nickel in perchloric acid.

In this investigation, in which an attempt was made to determine in perchloric acid medium the uranium derived from uranium concentrates of approximately 95 per cent. purity, the results were always high. As this error was greater than could be accounted for by the photometric error alone, other sources of error, particularly those due to the interfering effect of suspected impurities in the sample, were investigated.

These investigations have shown that unexpectedly large errors can arise from relatively small amounts of certain substances present as impurities. Hence, except for samples that are particularly suitable or of extremely high purity, these chemical errors are likely to exceed the photometric or physical errors to the extent that the often quoted accuracy of the difference method can be very misleading.

In view of the fact that difference photometric methods are frequently used for the assay of elements of relatively low extinction coefficient, the common mineral acids being used as the media, in which a large number of elements can be a source of interference, it is thought that a paper of this nature will draw attention to the magnitude of the errors to which at least one, and very likely more, of these systems is liable. Moreover, in showing the limitations of the method, the paper will help the analyst to decide whether the method will give results of the accuracy required for his particular samples.

Uranium has been determined by difference spectrophotometry in a sulphuric acid medium by Susano, Menis and Talbott,[7] Bacon and Milner[5] and Velthuis.[8] Silverman and Moudy[9] determined uranium in concentrations of 10 to 70 mg per ml in perchloric acid solution; the results shown in their paper appear to have been obtained by conventional photometric measurement, although they suggest that a technique resembling Bastian's difference procedure can be used as an optional method. Apart from stating that the perchlorates of aluminium (0·1 g per 25 ml), iron (0·2 g per 25 ml), thorium (1·0 g per 25 ml) and zirconium (0·2 g per 25 ml) do not interfere, no study was made of possible interfering substances. (It is shown later in this paper that iron does interfere with the determination.)

In the present study, perchloric acid medium was decided upon because the acidity does not have to be controlled to within the same narrow limits as sulphuric acid and because it was felt that the interference of substances such as iron and cerium would be far less serious. Perchloric acid could also be used for the direct attack of the uranium concentrates—organic matter was absent—and, at the same time, to oxidise the uranium to the sexavalent state.

Experimental

Apparatus and reagents—

A Beckman model DU spectrophotometer with a photomultiplier attachment was used for the investigation. A setting of 3 on the photomultiplier gave adequate stability to the meter needle and permitted the use of a relatively narrow slit.

The uranium oxide used for the standard solutions was prepared from crude uranium oxide by a cellulose - alumina column procedure, which normally yielded a product of approximately 99·95 per cent. purity. An assay of this material by a gravimetric method in which a cellulose - alumina column was used for the separation of impurities[10] showed a purity of 99·9 ± 0·1 per cent. Each individual standard solution was prepared from weighed portions of this oxide.

Temperature effects—

The effect of temperature differences between the reference and sample solutions on the optical density of the uranyl perchlorate in perchloric acid medium was investigated.

A solution of uranyl perchlorate equivalent to 1·74 g of U_3O_8 in 50 ml of 30 per cent. v/v perchloric acid was measured against itself in 2-cm cells and then the temperature of the solution in one cell was raised and the measurement repeated. Several relative optical-density readings were taken for various temperature differences between the reference and sample solutions. The relationship between the relative optical density and the temperature difference between the reference and sample solution from 0° to 11° C was linear. An increase of 1° C in the temperature of the sample, above that of the reference solution, caused an increase in relative optical density of approximately 0·004. For the conditions described under "Method," the temperature difference between the reference and the sample and standard solutions should not exceed 0·5 °C for a precision of ±0·1 per cent.

This requirement was easily satisfied by making up the standard and sample solutions to just below the mark in calibrated flasks, and then mixing and setting them aside overnight beside the spectrophotometer. The solutions were made up to the mark with water that had also stood beside the instrument overnight and the optical densities were then measured with as little delay as possible. The cells were handled by the neck while they were being filled and both reference and sample solutions were inserted simultaneously into the compartment. The reference cell was removed from the compartment while the sample cell was being filled with the next solution. To minimise heating of the compartment, an insulating layer of cotton-wool was placed between it and the lamp housing and, except while readings were being taken, the lid of the compartment was left off. A test showed that, when the reference cell was repeatedly inserted into the compartment for triplicate measurement of four sample solutions, the temperature of the reference solution rose 0·4° C above that of the sample solution. In all subsequent measurements, the reference solution was replaced by a fresh portion from the flask after every fourth sample.

SELECTION OF WAVELENGTH AND OF PERCHLORIC ACID CONCENTRATION—

Three 0·85-g portions of uranium, as U_3O_8, were dissolved in 10, 15 and 20 ml, respectively, of 70 per cent. perchloric acid and diluted to 50 ml. The optical densities of these solutions were measured at wavelengths from 400 to 435 mμ in 1-cm cells against reference solutions containing 10, 15 and 20 ml, respectively, of perchloric acid. A plot of these readings is shown in Fig. 1. A solution containing 20 ml of perchloric acid per 50 ml does not show absorption in this region of the spectrum.

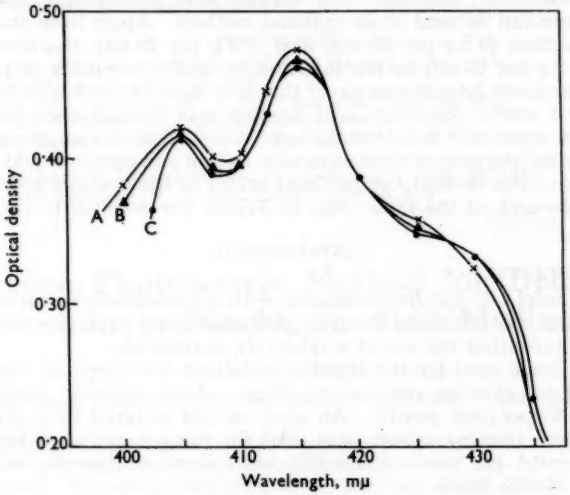

Fig. 1. Absorption spectra in 1-cm cells of uranyl perchlorate (as 0·85 g of U_3O_8 per 50 ml) in perchloric acid solutions of different concentrations measured against a perchloric acid reference solution at a theoretical half-intensity band-width of 0·29 to 0·33 mμ: curve A, 10 ml of perchloric acid per 50 ml; curve B, 15 ml of perchloric acid per 50 ml; curve C, 20 ml of perchloric acid per 50 ml

Although absorption is a maximum at 415 mμ, measurement at this wavelength would require fairly close control of the acidity of the solutions—a condition that would be difficult to meet as the perchloric acid is heated to the appearance of fumes during dissolution of the sample. At 418 to 420 mμ the optical density is unaffected by the variation of acid concentration, at least within the range 10 to 20 ml of perchloric acid per 50 ml of solution. A concentration of 15 ml of perchloric acid per 50 ml of solution and a wavelength of 420 mμ were adopted as standard in all tests.

CONCENTRATION OF THE REFERENCE SOLUTION—

The procedure used and its theoretical justification have been given elsewhere.[1,6] To obtain the results shown in Table I, amounts of uranium oxide, as U_3O_8, from 0 to 4·5 g at 0·5-g intervals were dissolved in 15 ml of perchloric acid and diluted to 50 ml. Each solution was measured against the next one of lower uranium content in the series, the solution of lowest uranium content being measured against the blank. The slopes of the graphs of relative optical density against uranium concentration (S in Table I) were calculated on the assumption that the graphs are linear. The optimum concentration of U_3O_8 in the reference solution is that for which the product of the slope and the amount of U_3O_8 in grams (S × G in Table I) is a maximum.

Although the optimum concentration for the 2-cm cell (with the photomultiplier sensitivity set at 3) is 3 g of U_3O_8 per 50 ml of solution, it was decided to use 2 g; there is a small sacrifice in accuracy, but, as the slit width is approximately one-third of that required for the 3-g reference solution, there is less interference from other substances. The narrower slit settings required when the photomultiplier sensitivity was set at 3 indicate the advantage of the photomultiplier attachment for this work.

TABLE I

DETERMINATION OF THE OPTIMUM CONCENTRATION OF THE REFERENCE SOLUTION

Measurements were made with a Beckman DU spectrophotometer in 2-cm cells at 420 mμ. Each solution contained 15 ml of perchloric acid per 50 ml

Concentration of uranium, as U_3O_8, in solution used to set scale to zero (G), g per 50 ml	Concentration of uranium, as U_3O_8, in solution used to obtain reading, g per 50 ml	Slit width, mm	Relative optical density (A)	Slope (dA/dG) (S)	S × G
With photomultiplier sensitivity set at 1—					
0	0·5	0·12	0·436	0·87	—
0·5	1·0	0·22	0·436	0·87	0·44
1·0	1·5	0·47	0·431	0·86	0·86
1·5	2·0	0·64	0·428	0·86	1·28
2·0	2·5	1·05	0·407	0·81	1·62
2·5	3·0	1·43	0·344	0·69	1·73
3·0	3·5	1·73	0·255	0·51	1·53
3·5	4·0	1·95	0·225	0·45	1·58
With photomultiplier sensitivity set at 3—					
0	0·5	0·026	0·436	0·87	—
0·5	1·0	0·045	0·436	0·87	0·44
1·0	1·5	0·080	0·431	0·86	0·86
1·5	2·0	—	0·431	0·86	1·29
2·0	2·5	0·24	0·411	0·82	1·64
2·5	3·0	0·40	0·390	0·78	1·95
3·0	3·5	0·64	0·329	0·66	1·97
3·5	4·0	0·94	0·262	0·52	1·82
4·0	4·5	1·20	0·191	0·38	1·52

INTERFERENCE OF OTHER SUBSTANCES—

The following elements were boiled with 15 ml of perchloric acid, diluted to 50 ml with water and the colours of the resulting solutions noted: aluminium, bismuth, cadmium, calcium, cerium, chromium, cobalt, copper, gold, iron, lead, magnesium, manganese, mercury, molybdenum, nickel, osmium, palladium, platinum, rhodium, selenium, silicon, silver, thorium, tin, titanium, tungsten, vanadium, zinc and zirconium. The absorption spectra for those elements forming coloured solutions with perchloric acid are shown in Fig. 2. The spectrum of ceric perchlorate was not plotted, because it can be readily reduced to the colourless cerous state by addition of a drop of hydrogen peroxide; molybdenum, palladium and platinum were neglected because only much larger amounts than are normally likely to be associated with uranium would cause any significant interference.

The approximate percentage of the element on a 2-g sample that would give a 0·1 per cent. error in the determination of uranium was calculated from the data in Fig. 2 and the results are shown in Table II. These figures are only approximate, because it is assumed in the calculation that the relationship between optical density and concentration is linear.

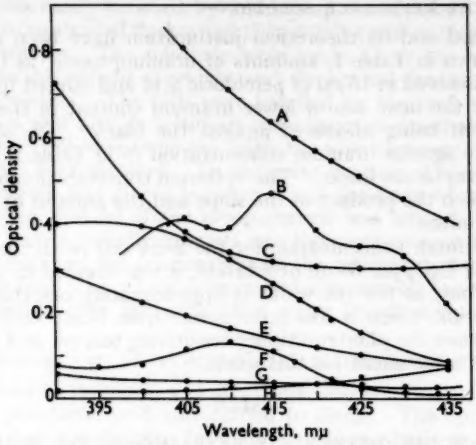

Fig. 2. Absorption spectra in 2-cm cells of some elements in perchloric acid solutions. All solutions, including the reference solution, contained 15 ml of perchloric acid per 50 ml and the theoretical half-intensity band-width was 0·25 to 0·57 mμ: curve A, 0·1 g of vanadium per 50 ml; curve B, 0·36 g of uranium per 50 ml; curve C, 2 mg of chromium, as Cr^{6+}, per 50 ml; curve D, 0·1 g of nickel per 50 ml; curve E, 8·95 mg of gold per 50 ml; curve F, 0·5 g of iron per 50 ml; curve G, 2 mg of chromium, as Cr^{3+}, per 50 ml; curve H, 0·1 g of cobalt per 50 ml

NOTE—From 390 to 440 mμ, 0·1 g of copper per 50 ml did not absorb

TABLE II

INTERFERENCE OF ELEMENTS ON THE DETERMINATION OF URANIUM

Element	Approximate amount of element to give error of 0·1 per cent. on a 2-g sample, %
Chromium^{6+}	0·00065
Chromium^{3+}	0·006
Gold	0·0075
Vanadium	0·017
Nickel	0·05
Cobalt	0·28
Iron	1·1

The figure given for chromium will be lower, because, on dilution of the perchloric acid after the solution has been boiled, a small amount is reduced to the less absorbent tervalent state.

The introduction of a drop of hydrogen peroxide and then boiling to destroy the excess will convert all the chromium to the tervalent state, but, nevertheless, the interference is still serious. The addition of hydrogen peroxide also reduces any gold to the metallic state, which allows the gold to be removed by filtration.

The effect of chromium, vanadium and nickel on the determination of uranium was tested in a more direct manner by adding various amounts of these elements to 1·84 g of U_3O_8, boiling with perchloric acid, diluting with water and measuring the relative optical density in the way used for the concentrate samples of uranium oxide (see "Method"). The amount of uranium equivalent to the weight of the element added is given in Figs. 3 and 4.

As in the previous experiment with chromium, the results are variable when the chromium is added before boiling with perchloric acid (see Fig. 3), but more consistent when the chromium is introduced as the dichromate after boiling with perchloric acid and dilution with water. The curve thus gives the maximum interference that can be expected from chromium. Curve B shows the effect of chromium when reduced to the tervalent state by the addition

of hydrogen peroxide after boiling with perchloric acid and dilution with water. This curve confirms the figure for tervalent chromium shown in Table II. The curves for vanadium and nickel also agree with the figures in Table II.

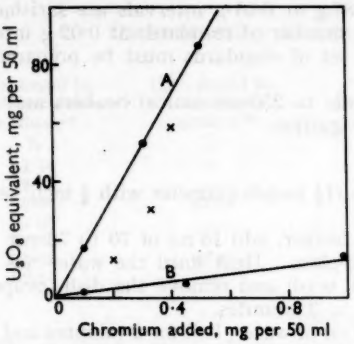

Fig. 3. Effect of chromium on the spectrophotometric determination of uranium at 420 mμ in 2-cm cells and with a slit width of 0·17 mm: curve A, chromium, as Cr^{6+}, added after heating to fumes with perchloric acid; curve B, chromium, as Cr^{3+}, added after heating to fumes with perchloric acid. The points × are for chromium added before heating to fumes with perchloric acid

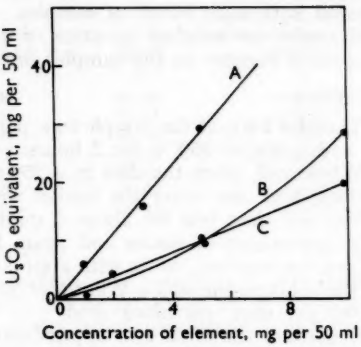

Fig. 4. Effect of vanadium, nickel and phosphorus on the spectrophotometric determination of uranium at 420 mμ in 2-cm cells and with a slit width of 0·17 mm: curve A, vanadium; curve B, phosphorus, as PO_4^{3-}; curve C, nickel

No curve has been drawn for iron, as the readings obtained were not reproducible with sufficient accuracy. A possible explanation is that the concentration of the perchloric acid has a pronounced effect on the optical density of the ferric perchlorate. As a rough guide, 50 to 100 mg of iron, as Fe, are equivalent to 5 mg of U_3O_8. It is of interest to note that Bastian[6] also found inconsistent optical-density readings for iron in perchloric acid.

The effect of sulphate is to increase the optical-density reading; 1 mg of sulphate, as SO_4^{2-}, is approximately equivalent to 1·2 mg of U_3O_8. Phosphate has a more pronounced effect (see Fig. 4). The sulphate in the samples of uranium concentrate was readily removed by ignition in a furnace at 950° C for a period of 1½ to 2 hours. Tests showed that the average amount of sulphate left in a sample that originally contained 10 per cent. of SO_4^{2-} was 0·012 per cent.

When the sample is dissolved by boiling with perchloric acid, the silica that separates is removed by filtration. However, during the washing procedure some of the very fine particles pass through even the retentive Whatman No. 3 filter-paper. High relative optical-density readings are obtained owing to the scattering of the light by these fine particles. To minimise errors due to this source, a portion of the solution was filtered after it had been made up to volume. As the filter-paper was not washed, the solutions were noticeably clearer and the results for uranium were lowered by an average of 0·3 per cent. This difference was not due to absorption by the filter-paper, as both standard and samples were filtered at the same time. Further attempts to clarify the solution, *e.g.*, by centrifugation, were not successful, and it is thought that an error of about 0·1 per cent. still remained.

No study was made of the possible sources of error in the determination of uranium due to the presence of the rare earths other than cerium and lanthanum, as this information was provided in the recent publication of a series of absorption curves for the rare earths in *M* perchloric acid.[11] At a concentration of 1 g of the element per 50 ml of solution and at a wavelength of 420 mμ, praseodymium, europium, gadolinium, terbium, thulium and ytterbium showed no absorption and samarium, neodymium, dysprosium and erbium only slight absorption. Holmium would give a positive error, as its solution absorbs light strongly at 420 mμ.

Method

Preparation of the standard solutions—

The weights of U_3O_8 for the standard solutions will depend upon the approximate amount of uranium in the sample. For samples containing between 91 and 93 per cent. of U_3O_8, six standards containing from 1·82 to 1·87 g at 0·01-g intervals are satisfactory. To cover a wider range of concentration, the same number of standards at 0·02-g intervals may be used with little sacrifice in accuracy. A set of standards must be prepared and measured with each batch of samples.

Transfer the weighed amounts of uranium oxide to 250-ml conical beakers and treat in the same manner as the samples, but omit the ignition.

Procedure—

Transfer 2·0 g of the sample to a porcelain dish (1¼ inches diameter with ¼ inch vertical side) and ignite at 950° C for 2 hours.

When cool, place the dish in a 250-ml conical beaker, add 15 ml of 70 to 72 per cent. perchloric acid and cover the beaker with a watch-glass. Heat until the water has been expelled and then boil for about 5 minutes. Cool, wash and remove the dish, evaporate to the appearance of fumes and again boil for 2 to 3 minutes.

Cool the solution, dilute with approximately 20 ml of water, boil for 2 minutes and then filter the solution through a Whatman No. 3 filter-paper. Wash the filter-paper and residue with 0·2 per cent. perchloric acid.

Evaporate the filtrate to approximately 35 ml (not less than 30 ml), cool and transfer to a 50-ml calibrated flask. Dilute to a little below the mark with water, mix and set the solution aside, together with the standards and a wash-bottle of water, next to the spectrophotometer for at least 3 hours or preferably overnight.

Dilute the solution to the mark with the water, mix and filter the standards and samples through dry 9-cm Whatman No. 3 filter-papers. Fill the 2-cm reference and sample cells with the standard of lowest uranium concentration and measure the relative optical density of the solution in the sample cell at 420 mμ with the instrument set to zero against the reference solution and with the photomultiplier sensitivity set at 3. Remove both cells from the compartment, rinse the sample cell three times with the next solution and insert both sample and reference solutions into the compartment simultaneously. Handle each cell by the neck only, and, between measurements, leave off the lid of the cell compartment. Measure the sample and standard solutions alternately. Unless the lamp housing is water-cooled, replace the solution in the reference cell with fresh solution from the calibrated flask after every fourth solution has been measured.

Plot the relative optical-density readings on linear graph paper and read off the values for the sample solutions.

Results

Four solutions with added impurities and containing known amounts of uranium were prepared and the uranium was determined by the proposed method. The amounts of the substances added were kept below the threshold of interference and no special precautions were observed other than those that are normally taken in a routine laboratory.

The results, together with the compositions of the solutions, are shown in Table III.

Table III

Determination of uranium in synthetic solutions

Each solution contained 10 mg of calcium and aluminium, 5 mg of lanthanum and zirconium, 2·5 mg of zinc, 1·25 mg of iron, 1 mg of thorium, manganese, copper and bismuth, 0·25 mg of arsenic, 0·125 mg of cobalt, 0·1 mg of tungsten, 0·025 mg of nickel and phosphorus and 0·005 mg of vanadium

Uranium added, g	Uranium found, g	Difference, assuming a 2-g sample, %
1·8400	1·8415	+0·07
1·8400	1·8400	0
1·8500	1·8475	−0·13
1·8500	1·8495	−0·025

Seven uranium concentrates were assayed by the proposed method and the results were compared with those obtained by the gravimetric cellulose-column and the volumetric cupferron methods (see Table IV).

TABLE IV

DETERMINATION OF URANIUM IN URANIUM CONCENTRATES

U_3O_8 found by cellulose-column method,[10] %	U_3O_8 found by volumetric method,[10] %	U_3O_8 found by proposed method, %	Mean, %	Difference between spectrophotometric and cellulose-column method, %
92·74	—	92·92, 92·95	92·94	+0·20
92·92	—	93·13, 93·28	93·21	+0·29
92·62	92·68	93·04, 92·94	92·99	+0·37
92·93	—	93·12, 93·26	93·19	+0·26
92·66	—	93·17, 93·14	93·16	+0·50
91·22	92·27	91·61, 91·73	91·67	+0·45
92·14	—	92·40, 92·50	92·45	+0·31

DISCUSSION OF RESULTS

The results for the synthetic solutions are considered to be satisfactory, but those for the uranium concentrates are high. These high results can be accounted for by the accumulative error due to the presence of small amounts of the substances found to give positive errors, namely, chromium, vanadium, nickel and silica. For example, the chromium contents of the first three samples in Table IV are 0·0023, 0·0027 and 0·0023 per cent., respectively. Assuming that all the chromium is in the oxidised state, the positive errors due to this element would be 0·35, 0·41 and 0·33 per cent., respectively. However, as previously stated, some of the chromium will be in the less interfering tervalent state. Nevertheless, this chromium will account for a large proportion of the uranium error.

It is possible to determine these impurities and to correct for their effect on the uranium determination or, alternatively, to devise methods of separation of these undesirable substances, but then the spectrophotometric method would lose much of its simplicity and directness and could no longer be considered as a competitor of the accepted gravimetric and volumetric methods.

I thank Mr. J. Levin for his helpful criticism and advice.

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The Polarographic Determination of Amino Acids Containing Thiol and Disulphide Groups

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A method is described for the determination of amino acids containing thiol and disulphide groups with a cathode-ray polarograph. These compounds can be determined in denatured and deproteinised sera and in serum hydrolysates. The method has been used to follow the reaction between mustard gas and the amino acids. Penicillin can be determined after conversion to penicillamine.

The possible applications of the method to investigate the serological diagnosis of cancer and the therapeutic action of amino acids against chemical agents are suggested.

Amino acids containing thiol and disulphide groups have been determined by conventional polarography both directly[1,2] and by means of their catalytic waves in ammoniacal cobalt solutions.[3]

It has been found that, when the cathode-ray polarograph is used for the direct determination of these compounds, the method is less susceptible to interference from capillary-active substances than is the direct conventional polarographic method and more specific than are the catalytic waves in ammoniacal cobalt solutions. As various workers have used the determination of these amino acids for serological diagnosis and for the determination of penicillin,[4] the procedure described was applied to the determination of the amino acids in denatured and deproteinised sera and serum hydrolysates and to the determination of penicillamine. The method is rapid and suitable for routine analysis.

Supporting electrolytes of 0·1 *N* sodium hydroxide, phosphate buffer solution of pH 7·0, 0·05 *N* hydrochloric acid and 0·1 *N* perchloric acid were tried in preliminary experiments. The curves when 0·1 *N* sodium hydroxide was used were small and the method was insensitive. Chloride ions interfered with the curves when pH 7·0 phosphate buffer solution was used. Cystine, cysteine, glutathione, oxidised glutathione, homocystine and homocysteine all gave reproducible curves in 0·05 *N* hydrochloric acid and 0·1 *N* perchloric acid.

Method

Apparatus—

The instrument used was a K1000 cathode-ray polarograph manufactured by Southern Instruments Ltd. The theory of this intrument has been described by Randles[5] and the instrument itself by Davis and Seaborn.[6]

Preparation of calibration curves—

Solutions of the amino acid to be studied were prepared in 0·05 *N* hydrochloric acid and placed in a polarographic cell with a dropping-mercury electrode and a mercury pool electrode. The solution was de-oxygenated, and the electrodes were connected so that the dropping-mercury electrode was at a potential of opposite sign to that indicated on the START POTENTIAL dial and the sweep of potential made the mercury drop more positive during the sweep. The START POTENTIAL and the SENSITIVITY controls were set to give the curve on the screen (see Fig. 1), and the peak height and the sensitivity were read. A calibration curve for each amino acid was constructed by using solutions of known composition and plotting the peak height in μA against concentration. Linear calibration curves were obtained of the form—

$$i = k\mathrm{C}$$

where i is the peak height in μA, C is the amino acid concentration in μg per ml and k is an experimentally determined constant.

Procedure for determining amino acids in solutions of unknown composition—

The amino acid solutions were acidified with hydrochloric acid so that the acid concentration was 0·05 *N*. The resulting solutions were treated as described for the preparation

of calibration curves, the product of the peak height of the curve and the sensitivity was noted and the amount of amino acid present was calculated from a previously prepared calibration curve.

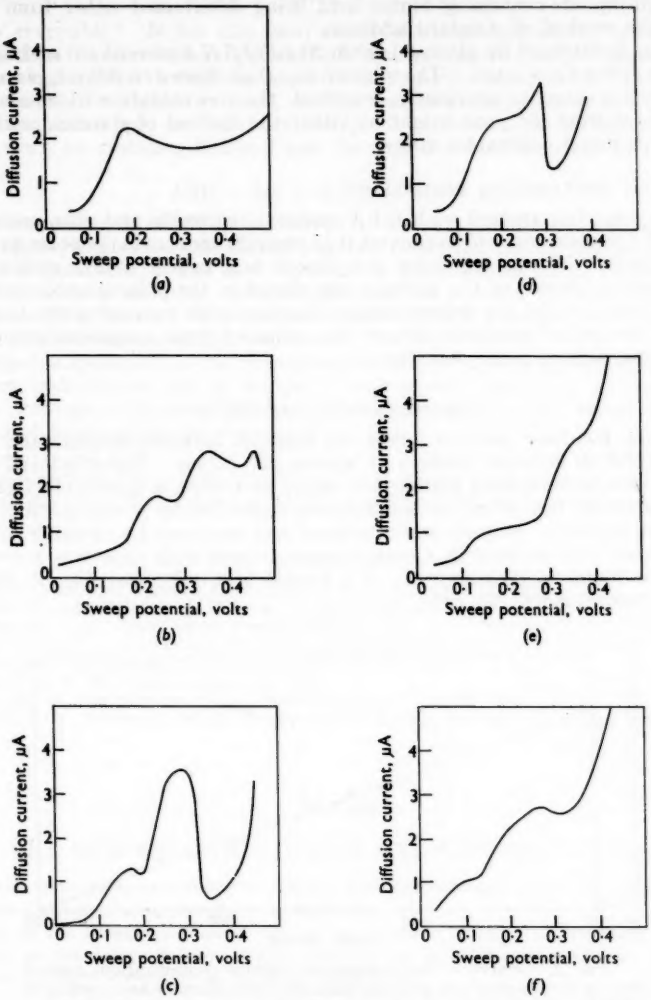

Fig. 1. Polarograms of solutions of amino acids in 0·05 *N* hydrochloric acid: (*a*), 4 μg of cystine per ml (start potential, −0·6 volt; sensitivity factor, 0·1); (*b*), 4 μg of cystine and 4 μg of cysteine per ml (start potential, −0·3 volt; sensitivity factor, 0·1); (*c*), 100 μg of homocystine and homocysteine per ml (start potential, −0·3 volt; sensitivity factor, 1·0); (*d*), 5 μg of homocysteine per ml (start potential, −0·4 volt; sensitivity factor, 0·1); (*e*), 5 μg of glutathione and oxidised glutathione per ml (start potential, −0·3 volt; sensitivity factor, 0·1); (*f*), 8·0 per cent. solution of denatured and deproteinated sera (start potential, −0·6 volt; sensitivity factor, 0·1)

PROCEDURE FOR DETERMINING AMINO ACIDS IN DENATURED AND DEPROTEINISED SERA AND SERUM HYDROLYSATES—

Serum was denatured and deproteinised by diluting 1 ml with 2 ml of distilled water, placing the solution in a boiling-water bath for 10 minutes, adding 2 ml of 20 per cent.

o-mercaptobenzoic acid solution and filtering the solution. Two millilitres of the filtrate were added to 3 ml of 0·05 *N* hydrochloric acid. The resulting solution was placed in a polarographic cell and treated as previously described for the determination of amino acids, the thiol or disulphide-containing amino acid being determined either from a calibration curve or by the method of standard addition.

Serum was hydrolysed by placing 1 ml in 20 ml of 5 *N* hydrochloric acid and boiling the solution under reflux for 8 hours. The solution was then diluted to 200 ml, placed in a polarographic cell and treated as previously described, the concentration of amino acid present being determined from the peak height by either the method of standard addition or from a previously prepared calibration curve.

PROCEDURE FOR DETERMINING PENICILLIN—

Penicillin was first treated with 0·1 *N* sodium hydroxide and then warmed with an excess of 0·1 *N* hydrochloric acid to convert it to penicillamine, as in the polarographic method of Dr. J. E. Page.* The penicillamine was diluted to a known volume with 0·05 *N* hydrochloric acid and an aliquot of the solution was placed in the polarographic cell and treated as previously described for the determination of amino acids containing thiol and disulphide groups. The amount of penicillin present was obtained from a calibration curve prepared from known amounts of pure penicillin.

APPLICATION OF THE METHOD

The method has been used to follow the reaction between cysteine and the nitrogen mustard gas HN2 in aqueous solution at known pH values. The methylbis(2-chloroethyl-amine (HN2) and cysteine were placed in a vessel containing a saturated-calomel electrode and a glass electrode; the pH of the solution was controlled by a Pye automatic titrimeter. At known time intervals, aliquots were removed and analysed for cysteine content by the proposed method; the decrease in cysteine concentration with time was noted. The rate of decrease was found to be kinetically of a pseudo first order (see Fig. 2), the rate being dependent on temperature and pH.

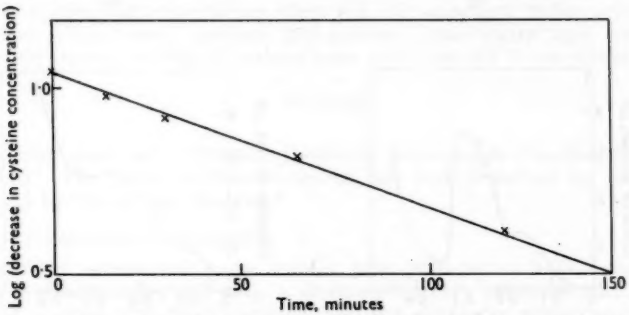

Fig. 2. Curve of log(decrease in cysteine concentration) against time for the reaction between methylbis(2-chloroethyl)amine and cysteine at pH 7·4

RESULTS

The thiol and disulphide-containing amino acids and penicillamine gave linear calibration curves that passed through the origin. The particular instrument used had a sensitivity of 1 μA of peak height equivalent to 27·5 μg of amino acid per ml for cystine, and a range of 1 μg to 1 mg per ml could be determined. Similar results were obtained for cysteine, homocysteine, homocystine, glutathione, oxidised glutathione and penicillamine. It was found to be possible to determine a thiol-containing and a disulphide-containing amino acid in the same solution, but it was not possible to resolve two thiol-containing amino acids, even when the derivative circuit of the instrument was used.

* Contribution to discussion on papers on assay of penicillin, *Analyst*, 1948, **73**, 197 to 216.

Discussion of Results

Classical potential measurements on solutions of cystine have shown that the reaction—

$$RSSR + 2H^+ + 2e \rightarrow 2RSH$$

is not strictly reversible.[1] It has also been shown that the cathode-ray polarograph loses less sensitivity than the conventional polarograph[7] and so is more sensitive for this determination; it is more convenient in that the maxima that usually occur in the cystine curve[1] do not occur when this instrument is used, so that maximum suppressors are not necessary. Similar considerations apply to the other disulphide-containing amino acids.

In their work on cysteine, Kolthoff and Barnum obtained evidence for the reversible reaction—

$$RSH + Hg \rightleftharpoons RSHg + H^+ + e$$

and they attributed the false diffusion current of about 1 μA, which they found at intermediate pH values, to an insoluble film of mercury cysteinate on the mercury drop. When the cathode-ray polarograph was used, cysteine in a phosphate buffer solution of pH 7·0 showed no similar false diffusion current; this may be because the speed of the voltage sweep does not allow mercury cysteinate to form. Similar considerations probably apply to the other thiol-containing amino acids.

The method is applicable to the determination of thiol and disulphide-containing amino acids in serum hydrolysates and in denatured and deproteinised sera; it may therefore be of use in the serological diagnosis of cancer, for which the catalytic waves in ammoniacal cobalt solutions[1] have previously been used.

The possible therapeutic action of thiol and disulphide-containing amino acids against chemical agents such as nitrogen mustards may be studied by following the decrease in amino acid concentration when the amino acid and the agent are allowed to react either in solution or *in vivo*.

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Notes

THE DETERMINATION OF ZINC OXIDE IN ZINC POWDER

In the course of an investigation into the properties of sprayed zinc films, a method for determining the zinc oxide content of zinc powder was required.

A survey of the literature showed that several indirec methods have been proposed, all of which depend on the determination of zinc metal in the powder by utilising its reducing power. The zinc oxide content is then determined by difference.

Tolley, in unpublished work, determined the metallic zinc content of powder samples by three methods. These were (*a*) conversion to nitrate, ignition to oxide and weighing, (*b*) dissolution in dilute acid and measurement of the volume of hydrogen evolved, and (*c*) dissolution in acid, conversion of the hydrogen to water and measurement of the weight of water formed. He obtained good agreement between the three methods, and determined the oxide content of zinc powder samples with a knowledge of their principal impurity contents, ferric oxide and silica, determined independently.

At least two direct methods for determining zinc oxide have been proposed. These depend on the relative solvent action on zinc oxide of neutral ammoniacal solutions. In the first method, developed by Tambon[1] and mentioned by Mellor and Thompson,[2] a solution of ammonia, ammonium carbonate and ammonium chloride is used. Another method, that of Osborn,[3] makes use of ammonium acetate solution. In both methods the sample is shaken or stirred with the appropriate solution, and, after separation of solid and liquid, the zinc oxide content is determined

either by measuring the weight loss of the dried residual zinc powder or by determining the zinc content of the solution by standard methods.

For the present work it was considered that a direct method for determining oxide content would be preferable. Such a method would be more accurate than an indirect method, since the oxide content of powders is generally only of the order of 2 to 10 per cent. and errors in the determination of metallic zinc would therefore be magnified when the results were used to calculate the oxide content by difference. Further, a complete analysis of the powder is required when any of the indirect methods is used, and also the apparatus and techniques used in these methods are generally complex.

It was decided to try Osborn's method[3] for the selective dissolution of zinc oxide, since this is the simplest and most recent of the direct methods. Analytical-reagent grade zinc powder was used as a standard sample for experiments. It was assumed that the powder would contain only zinc and zinc oxide, the latter being automatically formed on the surface of zinc exposed to the atmosphere. Since no significant impurities were likely to be present in this powder, its oxide content could be calculated from the results of an accurate gravimetric determination of zinc.

Experimental

Determination of the total zinc content of zinc powder and hence the oxide content—

A standard solution was prepared by dissolving a weighed amount of analytical-reagent grade zinc powder in dilute nitric acid, evaporating the solution almost to dryness with a calculated amount of dilute sulphuric acid, re-dissolving with the addition of the necessary amount of sulphuric acid and diluting to standard volume in a calibrated flask. Aliquots of the solution were taken with a calibrated wash-out pipette, and the zinc was precipitated as oxinate (zinc 8-hydroxyquinolinate) from an acetic acid - ammonium acetate medium in accordance with standard analytical procedure.[4] The precipitate was collected and weighed in a sintered-glass crucible.

The mean of several determinations of the total zinc content of the powder was 99·19 per cent., which corresponds to a zinc oxide content of 4·1 per cent.

Determination of oxide content by Osborn's procedure—

Preliminary experiments were carried out by stirring a weighed amount of sample (about 2 g) with 100 ml of 30 per cent. ammonium acetate solution in a beaker for 10 minutes, after which the beaker was set aside for 2 hours as described in the original method. The residual solid was transferred to a sintered-glass crucible, washed with absolute ethanol, dried by suction and weighed. Difficulty was experienced in the quantitative transference of the solid from beaker to crucible and the results were erratic, probably because drying was incomplete. This approach was abandoned in favour of a direct gravimetric determination of zinc in the separated solution. A series of experiments was carried out in which weighed amounts of sample were stirred with ammonium acetate solution as before. After being set aside, the mixture was passed through a filter-paper. The residue was washed with water and the washings were added to the filtrate. Zinc was determined in the filtrate by precipitation as oxinate and the apparent oxide content of the sample was calculated. Typical results were as follows—

Weight of zinc taken, g	0·8771	0·9040	1·0953	1·0661
Weight of zinc dissolved, g	0·0462	0·0492	0·0707	0·0617
Calculated zinc oxide content, % ..	6·6	6·8	8·0	7·2

Instead of the 30 per cent. solution recommended by Osborn, a 12 per cent. ammonium acetate solution was used. This change in concentration allowed the final ammonium acetate concentration to be easily adjusted to correspond with standard conditions for the precipitation of zinc oxinate.

The results show a considerable spread, and, by comparison with the figure of 4·1 per cent. of oxide found by accurate gravimetric determination of zinc in zinc powder, indicate that in Osborn's method both metallic and combined zinc are dissolved.

Separation by removal of metallic zinc—

Several unsuccessful attempts were made to remove metallic zinc from the powder sample and leave the zinc oxide in a suitable state for dissolution in ammonium acetate. The methods tried were (*a*) treatment of a suspension of the powder in water with acid-free ferric sulphate,[5] (*b*) treatment of the powder with cupric sulphate solution with subsequent separation of the oxide from the precipitated copper, and (*c*) amalgamation of the metallic zinc.

With methods (*a*) and (*b*) it was found that zinc oxide also dissolved. With method (*c*) metallic zinc did not completely amalgamate with mercury even at a temperature of 100° C.

Method

Treatment of zinc powder with ammonium acetate solution in absence of air—

An apparatus was devised in which to carry out Osborn's ammonium acetate procedure and subsequent filtration in the absence of air (see Fig. 1). This consists of a 250-ml Buchner flask with a short length of rubber pressure tubing fitted with a screw clip attached to the side-arm. The neck of the flask is fitted with a rubber bung through which passes a glass tube that terminates near the flask bottom in a No. 3 or 4 sintered-glass disc with an external diameter slightly less than the internal diameter of the neck of the flask. Outside the flask this tube has two right-angled bends and is closed with rubber pressure tubing and a screw clip. Zinc powder is treated with ammonium acetate solution in this apparatus as follows. A definite volume of ammonium acetate solution is introduced into the dry flask. The bung with the dry filter-tube is placed in the flask. The apparatus is evacuated through the side-arm by a vacuum pump until the solution just boils. The side-arm is then closed and nitrogen from a cylinder is slowly introduced through the filter-tube until bubbles almost cease to pass through the sintered-glass disc. The side-arm is then opened and a steady stream of nitrogen is allowed to bubble through the solution for 5 minutes. The zinc powder sample is weighed in a glass capsule and introduced into the apparatus by removing the rubber bung momentarily. The side-arm and filter-tube are then closed and the flask is shaken by hand and set aside for the appropriate time. For filtration, nitrogen pressure is applied through the side-arm and the filtrate blown through the filter-tube; aliquots of the filtrate can then be taken for zinc determination.

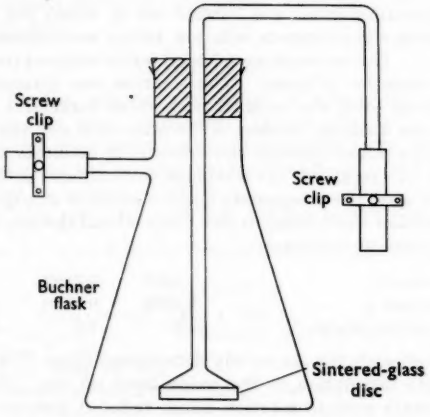

Fig. 1. Apparatus for the treatment of zinc powder with ammonium acetate solution in the absence of air

Results

A series of oxide determinations was carried out with this apparatus. Each sample was treated with 100 ml of ammonium acetate solution, and a 50-ml aliquot of the filtrate was taken for the gravimetric determination of zinc. The results obtained with 6 and 12 per cent. w/v solutions of analytical-reagent grade ammonium acetate are shown in Table I.

These results are reproducible with different samples of the same powder and are independent of the concentration of ammonium acetate solution between 6 and 12 per cent. They compare fairly well with the mean value of 4·1 per cent. obtained from the total zinc determination.

In an attempt to simplify the determination somewhat, a series of experiments was carried out without evacuation of the apparatus at the start of each determination. Nitrogen was allowed to bubble through the ammonium acetate solution for a few minutes before the sample

was added. The results were as follows, the zinc being determined on a 50-ml aliquot of the filtrate—

Weight of sample taken, g	1·7523	2·0128	1·9888	1·8730
Weight of soluble zinc found, g ..	0·0269	0·0288	0·0268	0·0273
Calculated zinc oxide content, % ..	3·9	3·6	3·4	3·6

The results, although of the right order, show a greater spread and have a lower mean value than those in Table I. Evacuation of the apparatus before the admission of nitrogen must permit a greater coverage of the surface of the powder by the reagent.

TABLE I

TREATMENT OF ZINC POWDER WITH AMMONIUM ACETATE SOLUTION IN ABSENCE OF AIR

In each instance zinc was determined on a 50-ml aliquot of the filtrate

Amount of zinc taken, g	Amount of zinc found, g	Calculated zinc oxide content, %
Zinc treated with 100 *ml of* 6 *per cent. w/v ammonium acetate solution—*		
1·7093	0·0275	3·8
2·0083	0·0289	3·6
1·8996	0·0284	3·7
1·9839	0·0295	3·7
Zinc treated with 100 *ml of* 12 *per cent. w/v ammonium acetate solution—*		
2·0780	0·0316	3·8
2·1195	0·0313	3·7
1·8619	0·0277	3·7
1·8020	0·0274	3·8

A further series of determinations was carried out in which the zinc powder was added to 50 ml of 12 per cent. ammonium acetate solution before evacuation. The reaction flask was then closed and evacuated. The contents were boiled under reduced pressure at room temperature for 15 minutes and set aside for 2 hours. The solution was filtered in the manner described previously, and the residual solid was washed with three successive portions of distilled water by removing the stopper and washing the stem of the filter-tube and the walls of the flask. During these washings, nitrogen was passed through the side-arm to maintain an inert atmosphere. After each washing the stopper was replaced, the washings were filtered in the usual way and added to the main filtrate. In this way, the ammonium acetate solution was quantitatively removed from the solid. The filtered solution was diluted to about 100 ml and the zinc determined gravimetrically as oxinate. The results were as follows—

Weight of zinc taken, g	1·1470	1·2992	1·2574	1·2359
Weight of zinc found, g	0·0393	0·0440	0·0420	0·0394
Calculated zinc oxide content, % ..	4·3	4·2	4·2	4·0

These results agree well with the accurately determined figure of 4·1 per cent. for the sample and indicate that complete dissolution of the oxide layer on zinc powder occurs if the sample is boiled with the ammonium acetate solution under reduced pressure.

Additional determinations were carried out in which the sample was introduced into the flask containing the reagent, but kept out of contact with the liquid until the flask had been evacuated. A special glass capsule, which could be tipped over by gravity when desired, was manufactured for this purpose. After they had been mixed, the contents of the flask were shaken for 10 minutes, set aside for 2 hours and filtered as before. Portions of 100 ml of 12 per cent. ammonium acetate solution were used, 50-ml aliquots of the filtrate being taken for the gravimetric determinations of zinc. Two determinations gave results of 4·0 and 4·1 per cent. for oxide content, which agree with the results shown above.

DISCUSSION OF RESULTS

The exclusion of air from the system when zinc powder is treated with ammonium acetate solution causes the proportion of zinc dissolved from the powder to be reduced from high variable values to a lower fairly constant value, *e.g.*, from 6 to 8 per cent. to from 4·0 to 4·3 per cent. for the sample studied. This must almost certainly be due to prevention of the oxidation of the surface of metallic zinc when exposed to air. Oxidation can occur when the surface oxide layer

on zinc powder is removed by a reagent such as ammonium acetate in the presence of air. The constancy of the figures obtained in the complete absence of air, even when the reagent concentration was varied, indicates that, under oxygen-free conditions, only zinc oxide is dissolved from zinc powder, the metallic zinc being unattacked by ammonium acetate.

The nature of the protective coating on particles of zinc powder is of some interest. It is known that, on continued exposure to a moist atmosphere, metallic zinc becomes coated with an impervious layer of basic zinc carbonate. The analytical-reagent grade zinc powder used in this work may also contain a similar compound formed by atmospheric exposure. If appreciable amounts of basic zinc carbonate are present, calculations based on the assumption that zinc powder contains zinc and oxygen alone are invalid, as also are determinations of oxid e content by the ammonium acetate procedure. The percentage of zinc in the surface compound can, however, be calculated from the total zinc determined gravimetrically and the amount of zinc soluble in ammonium acetate solution if it is assumed that the whole of the surface film is dissolved by the ammonium acetate treatment and that the zinc contains no other impurities.

If B is the percentage of zinc in the surface film compound and S and T are the percentages in the powder of zinc soluble in ammonium acetate solution and of total zinc, respectively, it can be deduced that—

$$B = \frac{100S}{100 + S - T}$$

Hence, B can be determined from experimental data. From the given experimental results, the average percentage of zinc soluble in ammonium acetate solution, S, is 3·4.

The total percentage of zinc in the powder, T, determined gravimetrically was 99·2.

Substitution in the equation gives a value for B of 80·4 per cent.

The theoretical percentage of zinc in zinc oxide is 80·33 per cent. It is therefore probable that the surface coating consists mainly of zinc oxide, since basic zinc carbonate would contain a much lower proportion of zinc.

Conclusions

It is concluded that, for the selective dissolution of zinc oxide in the presence of zinc by ammonium acetate solution, air must be absent. Zinc oxide can be satisfactorily determined in zinc powder by boiling the sample under reduced pressure at room temperature with 6 to 12 per cent. ammonium acetate solution and filtering the mixture in an atmosphere of nitrogen. Soluble zinc can then be determined by oxine precipitation.

It is hoped to extend the application of this method to samples of sprayed zinc film.

I thank Dr. G. Tolley for his advice in connection with this work.

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THE SEPARATION OF MANGANESE AS ITS DIETHYLDITHIOCARBAMATE COMPLEX IN THE PRESENCE OF CERIUM, AND ITS DETERMINATION

Because of their chemical similarity, manganese dioxide and cerium may be co-precipitated during the separation of cerium from the other lanthanons. In order to assess the efficiency of procedures for the purification of this precipitate, it is necessary to be able to determine manganese down to the parts per million level in the presence of cerium. The classical method of oxidation to permanganate with potassium periodate or persulphate simultaneously oxidises cerium to the ceric state, and, although the absorption peaks are widely separated, there is considerable interference with the measurement of the permanganate band at low manganese to cerium ratios.

In fact, it has been found to be impracticable to determine manganese directly in the presence of cerium below a ratio of about 1 in 2000.

Wyatt[1] has described the extraction of manganese with diethylammonium diethyldithiocarbamate in chloroform from acetate-buffered solutions. The manganese diethyldithiocarbamate can either be measured directly in the absence of interfering elements or by the permanganate colorimetric method after removal of the organic matter. We have found this method to be unreliable when applied to cerium compounds for two reasons. First, extraction is not complete at pH values less than about 6·5 in acetate medium (the aqueous solution in Wyatt's method buffers at about this pH or lower, leaving a degree of uncertainty in the extraction). Secondly, some samples have produced a foam with chloroform, which does not separate in any reasonable time. Citrate medium was tried instead, despite the statement[2] that manganese is not extracted from citrate solution at a pH of 7·5 to 8·5. We find that complete extraction of manganese is possible from 10 per cent. ammonium citrate solution at pH values between 7·0 and 8·5, and in this respect confirm the work of Bode.[3]

Cresol red appears to be a suitable indicator for the purpose; indicators changing to blue in alkaline solution are not recommended, since, in the presence of cerium, the aqueous layer tends to become yellow and the colour change of the indicator is masked.

Method

Reagents—

All reagents should be of recognised analytical grade.

Diethylammonium diethyldithiocarbamate solution—To 50 ml of chloroform add 3 ml of diethylamine and 1 ml of carbon disulphide. For use, dilute to 500 ml with chloroform.

Ammonium citrate solution, 50 per cent.—Add ammonia solution, sp. gr. 0·880, to 500 g of citric acid until the pH is 7 and then dilute with water to 1 litre.

Cresol red indicator solution—Dissolve 1 g of the solid indicator in 1 litre of 60 per cent. industrial methylated spirit.

Sulphuric acid, 10 N.

Nitric acid, 16 N.

Phosphoric acid, diluted (1 + 4)—Dilute 20 ml of 85 per cent. phosphoric acid to 100 ml with water.

Potassium periodate.

Procedure—

To the sample in a 100-ml graduated separating funnel add 10 ml of 50 per cent. ammonium citrate solution, a few drops of cresol red indicator solution and ammonia solution, sp.gr. 0·880, until the indicator just turns purple (pH 7·5 to 8·0). Dilute to 50 ml and shake vigorously until the solution is clear. Add approximately 10 ml of diethylammonium diethyldithiocarbamate

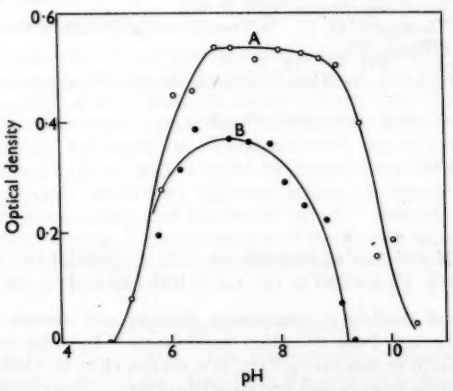

Fig. 1. Effect of pH on the extraction of manganese: curve A, solution containing 100 μg of manganese; curve B, solution containing 100 μg of manganese and 1 g of potassium cyanide

solution and shake vigorously for 1 minute. Allow the layers to separate and withdraw the lower organic layer into a 100-ml beaker. Repeat the extraction twice more, collecting the organic layers in the 100 ml beaker.

Add 5 ml of 10 *N* sulphuric acid to the combined extracts and evaporate gently until the chloroform has been removed. Add a few drops of concentrated nitric acid, cover the beaker with a watch-glass and evaporate the solution until fumes of sulphur trioxide appear. Cool the beaker somewhat, add 5 ml of diluted phosphoric acid (1 + 4), 10 ml of water and about 0·2 g of potassium periodate and then boil the solution for 5 minutes or until the appearance of a pink colour. Keep the solution hot for 10 minutes and then cool and dilute to 25 ml in a calibrated flask. Measure the optical density with an absorptiometer or spectrophotometer.

Results

Solutions containing 100 μg of manganese were adjusted to various pH values and extracted. The final permanganate colour was measured in 4-cm cells with a Spekker absorptiometer, the mercury lamp being used with Calorex, Ilford No. 605 and Wratten 77 filters, a combination that isolates the mercury line at 546 mμ. The results are shown in Fig. 1 (curve A); they indicate that complete extraction is possible between pH values of 7·0 and 8·5. These results are similar to those of Bode, who found extraction to be complete between pH values of 6 and 9.

Manganese was determined in spiked 5-g samples of a cerium nitrate known to contain a small amount of manganese. The results were as follows—

Manganese added, μg	Nil	20	40	60	80	100
Manganese recovered, μg ..	4·5	23·5	43·5	61·0	79·0	104·0

The positive difference between the manganese recovered and the manganese added indicates that the original sample contained manganese.

Interferences

Hydroxylamine and sulphur dioxide—Because of the statement by Bode that hydroxylamine and sulphur dioxide interfered, experiments were tried with hydroxyammonium chloride and sodium metabisulphite. It was found that 1-g amounts of these reagents caused only slight interference with the proposed method, and even boiling or prolonged standing in acid or alkaline solutions had relatively little effect; results with 100 μg of manganese were never lower than 94 μg. This accuracy is probably sufficient for most purposes.

Cyanide—Bode reported that cyanide caused no interference between pH values of 8 and 9. With our procedure we have found that 1 g of potassium cyanide causes interference over the whole pH range within which extraction takes place. The effect is shown in Fig. 1 (curve B).

Phosphate and pyrophosphate—A 1-g portion of sodium dihydrogen orthophosphate had no effect on the recovery of 100 μg of manganese; 1 g of potassium pyrophosphate reduced the recovery of 100 μg of manganese to 16 μg.

We are grateful to the Directors of Thorium Limited for permission to publish this Note and Mrs. M. P. Rose for experimental assistance.

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THE DETECTION OF COBALT AND NICKEL IN QUALITATIVE ANALYSIS

It was reported by Clark[1] that cobalt sulphide is soluble in pyridine containing toluene-3:4-dithiol (dithiol) to give a blue solution, whereas nickel sulphide is much less soluble, its colour reaction is masked by that of cobalt and is much less characteristic.

Morrison and Furst[2] have recently described quinoxaline-2:3-dithiol, which can be readily prepared, is very stable and, like 1:8-dimercaptonaphthalene,[3] gives an intense red colour with nickel in alkaline solution.

It has been found that nickel sulphide dissolves in an alkaline solution of quinoxaline-2:3-dithiol to give an intense red solution. Cobalt sulphide also dissolves, giving an orange-yellow solution, in which traces of nickel cannot be detected. However, in presence of an excess of sodium sulphide, only nickel sulphide dissolves.

These reactions can be used to detect the presence of both cobalt and nickel in the black precipitate of sulphides formed in group 4 of the usual analytical scheme, without the need for prior dissolution in acid. The reactions are given on gentle warming, both by the freshly formed precipitates and by precipitates that have been kept for several weeks, and they are not affected by the presence of zinc or manganese sulphides.

Ferrous sulphide, which is occasionally present in group-4 precipitates, dissolves in a solution of the zinc complex of toluene-3:4-dithiol (zinc dithiol) in pyridine, giving a pink or red solution. In large amounts, iron changes the blue of the cobalt complex to purple, but does not otherwise interfere. Ferrous sulphide is also soluble in a solution of quinoxaline-2:3-dithiol, giving an intense green colour, which is discharged when the solution is boiled, but does not interfere with the formation of the red nickel complex.

Method

Reagents—

Zinc complex of toluene-3:4-dithiol—Prepared as described by Clark.[4]

Quinoxaline-2:3-dithiol solution—A 0·1 per cent. solution in *N* sodium hydroxide containing 1 per cent. of sodium sulphide, $Na_2S.9H_2O$. This solution is stable for several weeks.

Procedure—

Precipitate the sulphides of the group-4 cations and wash the precipitate with dilute hydrochloric acid, as in the usual procedure.

Transfer minute specks of the black precipitate to—

(*a*) 0·5 ml of pyridine containing a trace (0·5 to 5 mg) of the zinc complex of toluene-3:4-dithiol and warm the solution. A blue colour indicates cobalt.

(*b*) 0·5 ml of quinoxaline-2:3-dithiol solution and bring to the boil. A red colour (with the eventual formation of a red precipitate) indicates nickel.

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PURITY TEST FOR METALLIC MERCURY USED IN POLAROGRAPHY

To prevent blockage of the dropping-mercury-electrode capillaries in polarographic work, only metallic mercury of the highest purity is used. The purity of the metal used in internal mercury (pool) electrodes need not be so high as that of the dropping electrodes. Nevertheless, the mercury should be sufficiently pure to prevent all interferences, since these limit polarographic work and preclude determinations of trace constituents. Interferences may result from the presence of either reducible or oxidisable materials, or impurities that may influence the physical properties of mercury and thereby affect the performance of the dropping-mercury electrode. A test for the suitability of mercury for polarographic work is therefore desirable.

Tests have been recommended by Müller,[1] Milner[2] and Wichers,[3] all of which depend on the observation of the physical behaviour of mercury. In the course of investigations of rapid polarographic determinations, some blank tests yielded surprisingly large currents. The magnitude of these currents was found to relate to the amount of impurities present in the mercury. A simple polarographic and polarometric method of testing the purity of mercury for use in dropping-mercury and pool electrodes was devised, based on this observation.

Method

Reagents—

Analytical-grade reagents and double-distilled water were used. Gelatin of ordinary grade was suitable. Mercury of highest purity obtained after cleaning with acid, triple-distillation and

de-greasing was used for the dropping-mercury electrode; the mercury sample under test was used for the mercury-pool reference electrode.

APPARATUS—

Recording polarograph—Polarographic determinations were performed with a Tinsley pen-recording polarograph, type MK/14, with an internal mercury electrode cell obtained from the same firm. The quicker 2-inch drive of the polarograph was used and the polarogram was recorded between zero and the decomposition voltage of each supporting electrolyte, with minimum damping and no counter current. Readings were made at a chosen voltage on the plateau of the polarogram from the zero current, slightly before the decomposition voltage of the supporting electrolyte. The zero current line was plotted by disconnecting the electrodes.

Polarometer—A simple home-made polarometer was constructed, similar to that described by Kolthoff and Lingane,[4] with a Hartmann-Braun galvanometer containing five sensitivity stages. Each scale division was 0·00397 μA at the highest sensitivity. The galvanometer deflection at a suitable fixed voltage was determined.

For each polarographic or polarometric determination, 10·0 ml of one of the following base solutions was added to a 20·0-g sample of the mercury to be tested in the pool electrode—

(*a*) a solution molar in ammonium hydroxide and ammonium chloride and containing 0·01 per cent. of gelatin and 2 per cent. of sodium sulphite;
(*b*) a solution molar in sodium hydroxide and containing 2 per cent. of sodium sulphite.

DISCUSSION OF RESULTS

Curve A, Fig. 1, is the polarogram of a purified mercury sample obtained with a molar ammonium hydroxide and ammonium chloride supporting electrolyte; it shows no initial wave (or a negligible one) from the zero current line. The subsequent current increase, resulting from increased applied voltage, represents only the residual current of the base solution. Curve B, Fig. 1, is the polarogram of an impure mercury sample with the same supporting electrolyte; it shows an initial sharp rise of a wave from the zero current line. Such a wave occurs whenever the mercury is impure.

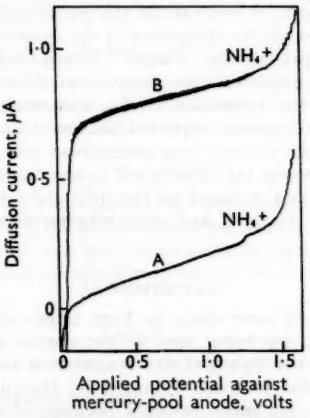

Fig. 1. Polarographic determination of the purity of mercury with a base solution molar in ammonium hydroxide and ammonium chloride and containing 0·01 per cent. of gelatin and 2 per cent. of sodium sulphite. The polarograms were recorded at room temperature: curve A, pure mercury; curve B, impure mercury

The galvanometer deflections, measured with the polarometer at a fixed voltage with both supporting electrolytes, indicated the degree of purity of the mercury samples. The lowest current

of $-0{\cdot}27$ μA, measured at an applied voltage of $-1{\cdot}3$ volts with a molar ammonium hydroxide and ammonium chloride supporting electrolyte, was obtained for a mercury sample of highest purity that was suitable for use in the dropping-mercury electrode. The galvanometer deflection for this sample served as a reference standard for other samples of mercury. Certain analytical-grade and ordinary-grade mercury yielded much higher current readings under the same conditions, which indicates lower purity. De-greasing of mercury by treating small batches with hot molar potassium hydroxide and then with acetone appeared to be of utmost importance for the purification of mercury. A suitable mercury for the dropping-mercury electrode can be obtained by cleaning with acid, double or triple-distillation and de-greasing. Cleaning with acid and de-greasing are generally adequate for mercury that is to be used for pool-electrode polarography.

I thank the Board of Directors of Israel Mining Industries for permission to publish this Note.

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THE SIMULTANEOUS SPECTROPHOTOMETRIC MICRO-DETERMINATION OF CALCIUM AND MAGNESIUM WITH ERIOCHROME BLACK T AND ETHYLENEDIAMINETETRA-ACETIC ACID

Eriochrome black T has been widely used as an indicator in the complexometric titration of magnesium and calcium.[1,2,3] Dirscherl and Breuer[4] and Harvey, Komarmy and Wyatt[5] have used the dye for the spectrophotometric determination of microgram amounts of magnesium, interference from calcium being avoided by its prior removal as oxalate or sulphate. Young, Sweet and Baker[6] have developed a method for the simultaneous determination of magnesium and calcium in a mixture, based on the difference in the stabilities of their complexes with Eriochrome black T at different pH values. Gasser[7] determined the two elements together by complexometric titration and completed their individual determination by the absorptiometric determination of magnesium after correction of the measured absorption for the contribution of calcium. Zak, Hindman and Baginski[8] reported the spectrophotometric titration of magnesium and calcium in spinal fluids; each element was determined after its separation from the other.

The method described permits the individual determinations of magnesium and calcium without their prior separation, and is based on the differences in the stabilities of the complexes of the metals with Eriochrome black T and with ethylenediaminetetra-acetic acid (EDTA) at the same pH.

Experimental

All absorption measurements were made in 1-cm Corex cells with a Beckman DU quartz spectrophotometer, a tungsten lamp being used as the source of radiation.

The absorption spectra of the dye and the magnesium complex solution at pH 9·5 were measured and found to be similar to those obtained by Harvey, Komarmy and Wyatt.[5] The wavelength 650 mμ was chosen for measurements because of the negligible absorption of the complex at this wavelength in comparison with that of the dye. Young, Sweet and Baker[6] have used this wavelength region in the determination of magnesium by measuring the decrease in absorption of the dye solution due to complex formation with magnesium.

Principle of the method

Curves A and B, Fig. 1, show the decrease in the absorption of a suitable concentration of the dye solution containing different amounts of magnesium and calcium, respectively, plotted against the corresponding amount of metal present. From these curves, the constants K_1 and K_2, which represent the decrease in the absorption of the dye solution due to complex formation with 1 μg of the corresponding metal, can be calculated. If the solution being studied contains a

mixture of magnesium and calcium, the measured decrease in the absorption, A, can be correlated to the respective metal concentrations by the relationship—

$$A = K_1X + K_2Y \quad .. \quad .. \quad .. \quad .. \quad .. \quad (1)$$

where X and Y are the corresponding amounts in micrograms of magnesium and calcium in solution.

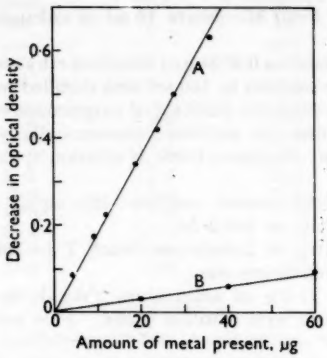

Fig. 1. Calibration curves for magnesium and calcium. Three millilitres of dye solution and 5 ml of buffer solution with different amounts of magnesium or calcium and diluted to 50 ml with distilled water: curve A, magnesium; curve B, calcium

The stabilities of calcium and magnesium ethylenediaminetetra-acetates (log K = 10·6 and 8·7, respectively) are such that, if an excess of the magnesium compound is added to a solution containing calcium, an amount of magnesium equivalent to the calcium will be liberated, according to the equation—

$$Ca^{2+} + MgR = CaR + Mg^{2+}$$

where R represents the organic radicle.

Hence, when an excess of magnesium ethylenediaminetetra-acetate is added to a second aliquot of the calcium solution, the decrease in the measured absorption, B, can be expressed as follows—

$$B = K_1 (X + 0{\cdot}607Y) \quad .. \quad .. \quad .. \quad .. \quad .. \quad (2)$$

the factor 0·607 being the magnesium equivalent of 1 μg of calcium.

Equation (2) is applicable only if the displacement reaction is quantitative under the experimental conditions. This assumption was proved to be valid by the results shown in Table I, in which the calculated and determined values of magnesium for a series of calcium concentrations are compared.

TABLE I

COMPARISON BETWEEN CALCULATED AND DETERMINED AMOUNTS OF MAGNESIUM

Amount of calcium present, μg	Calculated magnesium equivalent of calcium present, μg	Amount of magnesium found, μg	Error, %
20·0	12·2	12·2	0
24·0	14·6	14·8	+1·4
32·1	19·5	18·5	−5·1
40·1	24·3	24·3	0
48·1	29·2	28·0	−4·1
60·1	36·1	35·5	−2·7

Suitable aliquots of the working solution of calcium were placed in 50-ml calibrated flasks, 2 ml of magnesium ethylenediaminetetra-acetate solution were added and the amounts of magnesium liberated were determined by the proposed procedure.

Method

Reagents—

All reagents should be of recognised analytical grade.

Calcium stock solution, 0·01 M—Dissolve 0·10 g of calcium carbonate in the minimum amount of hydrochloric acid, heat to remove all carbon dioxide and dilute the solution to 100 ml with distilled water.

Calcium working solution, 0·001 M—Dilute 10 ml of calcium stock solution to 100 ml with distilled water.

EDTA solution, 0·01 M—Dissolve 0·3724 g of disodium ethylenediaminetetra-acetate dihydrate in distilled water and dilute the solution to 100 ml with distilled water.

Magnesium stock solution—Dissolve 0·2464 g of magnesium sulphate heptahydrate in 100 ml of distilled water and standardise the solution volumetrically with EDTA solution.

Magnesium working solution—Prepare a 0·001 *M* solution by suitable dilution of the magnesium stock solution.

Magnesium ethylenediaminetetra-acetate solution—Mix equal volumes of 0·01 *M* solutions of magnesium and EDTA and dilute to 0·001 *M*.

Dye solution—Dissolve 0·10 g of Eriochrome black T in 100 ml of distilled ethanol. This solution must be freshly prepared before use.

Buffer solution—Dissolve 17·5 g of ammonium chloride in 150 ml of ammonia solution, sp.gr. 0·880, and dilute to 250 ml with distilled water. Five millilitres of this solution, diluted to 50 ml, have a pH of 9·5.

Procedure—

Prepare duplicate samples of the mixture. To one solution add 5 ml of buffer solution and 3 ml of dye solution. Dilute to 50 ml, and, 45 minutes later, measure the absorption at 650 mμ with distilled water in the comparison cell. Measure the absorption of the reagent blank. The difference between the absorption of the reagent blank and that of the solution gives the value of A in equation (1).

To the other solution add 5 ml of buffer solution, 3 ml of dye solution and 2 ml of magnesium ethylenediaminetetra-acetate solution, and dilute to 50 ml with distilled water. Determine the difference between the absorption of this solution and that of the corresponding blank solution. This gives the value of B in equation (2). Use these values of A and B in equations (1) and (2) to calculate the magnesium and calcium concentrations.

Results

The experimental values of A and B for two mixtures of magnesium and calcium, together with the corresponding values of K_1 and K_2, are shown in Table II.

Table II

Application of the method

The decrease in the optical density of the dye solution corresponding to 1 μg of magnesium, K_1, is 0·01765; that of calcium, K_2, is 0·00144

Amount of magnesium present, μg	Amount of calcium present, μg	Value of A	Value of B	Amount of magnesium found,* μg	Amount of calcium found,* μg
12·2	20·0	0·227	0·420	11·2	20·8
12·2	20·0	0·227	0·430	11·0	21·9
24·3	12·0	0·437	0·555	23·7	12·7
24·3	12·0	0·445	0·565	24·2	12·9

* These values were obtained by substituting the values of K_1 and K_2 and the observed values of A and B in equations (1) and (2).

Table III shows the results of thirteen analyses of synthetic mixtures of calcium and magnesium. In experiments 1 to 4, the values for magnesium were obtained from equation (2) by direct substitution of the actual amounts of calcium added.

The results show that the method has an error of less than $\pm$10 per cent. of the individual determinations when the concentration of either element, in terms of magnesium, lies between 8 and 36 μg per 50 ml of solution.

TABLE III

ANALYSIS OF CALCIUM - MAGNESIUM MIXTURES

Experiment No.	Amount of calcium present, μg	Amount of calcium found, μg	Error, %	Amount of magnesium present, μg	Amount of magnesium found, μg	Error, %
1	12·0	—	—	12·2	11·8	−3·3
2	20·0	—	—	9·7	9·5	−2·1
3	32·1	—	—	14·6	14·5	−0·7
4	40·1	—	—	9·7	9·1	−6·2
5	8·0	8·2	+2·5	24·3	24·1	−0·8
6	12·0	12·8	+6·7	24·3	24·0	−1·2
7	16·0	16·2	+1·3	9·7	9·0	−7·2
8	20·0	21·1	+5·5	12·2	11·7	−4·1
9	24·0	22·0	−8·3	14·6	13·5	−7·5
10	28·1	26·0	−7·5	17·0	16·5	−2·9
11	28·1	28·4	+1·1	12·2	12·5	+2·5
12	32·1	33·0	+2·8	7·3	6·8	−6·8
13	32·1	31·8	−0·9	9·7	10·4	+7·2

CONCLUSIONS

The proposed method is of limited application, as all cations that form more stable complexes with EDTA than does magnesium cause a positive error in the calcium determination. Hence, if the method is to be applied to solutions containing such cations, they must either be removed by suitable separation procedures or suppressed, whenever possible, by auxiliary complexing agents. Nevertheless, the method could be applied to the analysis of biological materials such as cerebrospinal fluids.

We thank Mr. Ch. Venkateswarlu for useful discussion and Dr. V. T. Athavale for his help and guidance.

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Book Reviews

THE ANALYTICAL USES OF ETHYLENEDIAMINETETRAACETIC ACID. By FRANK J. WELCHER. Pp. xviii + 366. Princeton, N.J., New York, Toronto and London: D. Van Nostrand Co. Inc. 1958. Price $8.50; 64s.

Professor Welcher's book on the analytical applications of EDTA is cast in the same encyclopaedic mould as his previous work on organic analytical reagents; it cites 963 references. In many ways it is complementary to Schwarzenbach's "Complexometric Titrations," since it is written from a different view-point. The text is in the nature of a comprehensive review of the multitudinous applications of EDTA, and any shortcomings that the reader may find should be judged in the light of this fact, for it must be realised that the author has squared up to a truly Herculean task. The way in which it has been completed cannot fail to win admiration. Even so, it must not be assumed that the survey is complete even within its time limits. For example, the reviewer can think immediately of at least five "major" papers (three of them concerned with non-metals) that are not mentioned.

The scattered literature of EDTA is characterised, more than most perhaps, by inconsistencies and a failure of authors to make a truly scientific comparison of their newly devised procedures

with those already proposed by other workers. There are many of these indicators (the author has tabulated them under the names of the elements for which they may be used), but anyone new to the subject is faced with a bewildering choice. It is not always helpful to read the text carefully, because of the confusing claims made by the original workers and their failure to make comparisons. It is the experience of the reviewer and that of others, for example, that Solochrome black 6B (p. 37) and Solochrome dark blue BS (pp. 37 and 38) are superior to the classical Eriochrome black T (p. 33) and murexide (p. 61) indicators for the titration of magnesium and calcium, respectively. Phthalein complexone (p. 48) is capable of forming colours with several cations that are not mentioned, and indeed is much less selective than would appear. Calcium cannot be titrated in the presence of magnesium in the pH range 10 to 12 (p. 120). The pH must be kept very close to or slightly above 12 (magnesium titrates at pH 10). Indeed, this popular method rarely, if ever, gives the correct recovery for calcium even in the presence of relatively small amounts of magnesium. On p. 64 a double error is perpetrated in stating the original authorship of the 3:3′-dimethylnaphthidine ferro-ferri-cyanide indicator system and in claiming its first usage with EDTA.

However, these are minor flaws in a book that will be of the utmost assistance to all those who are engaged in analytical work in which EDTA may be used. The bulk of the text is concerned with the titrimetric applications of EDTA, but there are excellent sections dealing with colorimetry, polarography, inorganic qualitative analysis and the use of EDTA as a masking agent in conjunction with other organic reagents, such as 8-hydroxyquinoline. I have no hesitation whatsoever in predicting that this book will be a "best-seller" or in recommending it unreservedly.

T. S. WEST

SOLVENT EXTRACTION IN ANALYTICAL CHEMISTRY. By GEORGE H. MORRISON, Ph.D., and HENRY FREISER, Ph.D. Pp. xii + 269. New York: John Wiley & Sons Inc.; London: Chapman & Hall Ltd. 1957. Price $6.75; 54s.

The first thing that should be made clear about this book is that the title is rather misleading; it deals exclusively with the solvent extraction of inorganic salts and complexes and no attempt has been made to include the multifarious and increasingly important applications of this technique to the analysis of purely organic materials. Within these wisely imposed limitations the authors have produced a valuable guide book to a rapidly developing field; this they have done by greatly expanding existing reviews and by collecting and correlating the wealth of scattered information, so much of which has not found its way into recognised scientific journals and is known only to those *cognoscenti* who have had access to the publications of the English and American Atomic Energy Projects and to records of their extra-mural activities in universities.

The book is divided into four parts. The first (78 pages) deals with basic theory. In the opinion of the reviewer this is the least successful part and criticisms could certainly be levelled at points of detail and treatment; but it is quite adequate for an understanding of the practical separations that follow. The second part of the book (45 pages) describes methods of extraction, general techniques and apparatus, and various ways of determining analytically the concentration of the species that partitions.

The third section of the book (64 pages) classifies extraction systems under the headings of ion-association systems (*e.g.*, halides, nitrates, alkyl phosphoric esters, long-chain and polyamines and 'onium salts) and chelate systems (*e.g.*, oxinates, dithizonates and cupferrates). This part forms a valuable extension of existing reviews and brings their subject matter and bibliography up to date to 1956. The last part of the book (60 pages) is a collection of selected procedures for the solvent extraction of no less than 66 different elements—an excellent indication of the range and power of this technique. Practical details are necessarily limited, but generally adequate; full literature references are provided and there are useful indications of interferences by elements co-extracted. The book concludes with 4 pages devoted to the physical properties of typical solvents, an index listing elements with the various systems available for their solvent extraction, and a good subject index.

All workers in this field will be aware of the almost insuperable difficulties of making a coherent "story" out of the mass of disconnected and often discrepant observations that comprise much of the literature of solvent extraction. Although the subject will surely justify a more critical and thorough treatment before long, the authors are to be congratulated on the present volume, which will undoubtedly prove valuable to all analysts who are not yet using the techniques of solvent extraction.

H. IRVING

DISINFECTANTS: THEIR VALUES AND USES. By W. E. FINCH. Pp. 188. London: Chapman & Hall Ltd. 1958. Price 30s.

Mr. Finch has had many years of experience in the disinfectant industry, and his book is based on this experience. It is primarily a discourse on phenolic and quaternary ammonium type disinfectants, with some information added on hypochlorites.

It contains valuable information, much of which has not been published before, on the formulation and performance characteristics of Lysols, the chloroxylenol disinfectants and the various types of black and white fluids, including the influence of different phenols and soaps on their activities, and the significance of the various hydrocarbon carriers. It contains practical suggestions for the laboratory control of disinfectants, their analysis and assessment of stability. The activities of quaternary ammonium compounds, and their limitations in application, are dealt with from a practical standpoint with emphasis on their adsorption on fibres and their use in disinfecting blankets.

The Rideal - Walker test is put into its right perspective as a means of assessing the value of disinfectants, and other phenol coefficient tests are discussed, including the Use-Dilution Confirmation test. The author apparently prefers a modified Chick - Martin test for assessing his use-dilutions.

It is a pity that the information given is presented in such a diffused manner, as this makes the book not easy to read. One must also say that it is unfair of the author to state in his Preface that "an attempt has been made to give a balanced survey of all types of disinfectants . . ." and then to dismiss iodine in eight lines, mercurials in nine lines, formaldehyde in less than a page and so on. There is not a regular flow in the treatment of the various topics and there is a tendency to dodge from one aspect to another; it is disconcerting, for instance, in reading about the activities of phenolic substances adsorbed on woollen fibres, suddenly to come across a sentence on the photogenic properties of *Pseudomonas pyocyanea* (p. 117), likewise, sterilisation by heat (given only just over a page) is irrelevant.

Some of the Tables are frankly difficult to understand, several of the references are wrong and the subject index could be improved; for instance, skin disinfection is indexed on pp. 65, 74 and 75, but there is also a section headed "Skin Sterilisation" on p. 118. Some of the manufacturing instructions given in the last chapter are trivial and the glossary is superfluous. Neither is the book without its unconscious humour—"lysols . . . give faint opalescence in 1 per cent. Silvertown mains water" (p. 54), and "The water closet can be replaced by the chemical closet" (p. 150).

One cannot help but feel that the book has been written with certain specific disinfectant preparations in mind.

The print is clear, the book is easily handled and there are very few typographical errors, although one is significant—in Table 1.1, the Rideal - Walker strain of *Salmonella typhi* should be N.C.T.C. 786, not 781.

The book should prove to be of value to all those concerned in the manufacture and control of disinfectants.

G. SYKES

PROGRESS IN STEREOCHEMISTRY. Volume II. Edited by W. KLYNE, M.A., D.Sc., Ph.D., and P. B. D. DE LA MARE, Ph.D., D.Sc. Pp. viii + 323. London: Butterworths Scientific Publications; New York: Academic Press Inc. 1958. Price 50s.; $8.80.

The well chosen volumes in Butterworths's new "Progress Series" are proving a boon to all analysts faced with the problem of keeping abreast—or at least in touch—with advances in other fields and the most recent developments in theoretical chemistry. The present Volume II of *Progress in Stereochemistry* reviews the position to the close of 1956 and devotes chapters to the stereochemistry of homolytic processes, displacements at unsaturated centres, the elements of Group V, and inorganic molecules and complex ions. It also reviews the importance of steric factors in crystallography, in mesomerism, and in immunochemistry, and includes an account of optically labile compounds.

H. IRVING

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